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STOCK ENHANCEMENT OF LOCAL POPULATIONS OF BLACKLIP ABALONE (*HALIOTIS RUBRA* LEACH) IN NEW SOUTH WALES, AUSTRALIA.

Thesis submitted by

Rowan C. Chick BSc (Hons)

in February 2010

for the degree of Doctor of Philosophy in the

School of Marine and Tropical Biology

James Cook University

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ABSTRACT

This thesis examines the release and long-term (>2 years) survival and growth of hatchery-reared larval and juvenile blacklip abalone (*Haliotis rubra* Leach), on natural coastal reefs in New South Wales (NSW), Australia. Abalone are demersal, relatively sedentary, marine molluscs, that support important commercial, recreational and indigenous fisheries in numerous locations around the world. This thesis was developed in response to substantial depletions of local populations of *H. rubra* along >250 km of the NSW coast and the ineffectiveness of traditional fisheries management strategies to arrest these declines. These failures stem from demographic processes, common to haliotids, that limit their ability to re-establish populations that have been subject to substantial decline. A series of laboratory and field experiments were designed and conducted to examine a range of factors, and their interactions, that can have substantial affects on the success of releasing hatchery-reared *H. rubra* to natural reefs. The principal finding was that successful stock enhancement of local populations can be achieved, and the greatest value of a stock enhancement strategy is likely to be gained where the primary management objective is rebuilding depleted natural populations. Methodology, baseline targets and other recommendations are provided that would aid implementation of a stock enhancement management strategy to complement current traditional fisheries management approaches. The objectives of the research in this thesis were to: 1) investigate factors affecting the settlement, metamorphosis and early growth of *H. rubra* larvae; 2) batch-tag larvae and juveniles to enable their identification when recaptured; 3) develop and test methods for the successful release of larvae and juveniles; 4) develop a monitoring strategy to estimate the abundance of released abalone through time; 5) quantify long-term survival and growth to provide minimum targets for stock enhancement; 6) determine the impact of releasing juveniles on wild populations; 7) provide a bio-economic analysis and; 8) provide recommendations for the implementation of a stock enhancement management strategy for *H. rubra* in NSW.

The release of larvae to natural reefs requires them to be exposed to a number of handling and transport processes. In laboratory experiments conducted in this thesis, greater proportions (commonly >75%) of larvae settled, metamorphosed and grew to larger sizes when exposed to settlement substrata for longer periods of time (>24 hours). There was a lower settlement response in the presence of water flow, although the addition of gamma-aminobutyric acid (GABA) increased the proportion of larvae that settled within short periods of time, i.e. 25 - 100% more in ≤60 sec.. Larvae were resistant to simulated handling and transport processes, indicating their utility for stock enhancement.

The tagging of larvae and juveniles is fundamental to assessing the success of an enhancement program. Hatchery-reared *H. rubra* larvae and juveniles were successfully batch-tagged. The

tagging procedures provided an indelible mark, enabling the identification of individuals once recaptured, and unambiguous differentiation from wild conspecifics. Successful batch-tagging was also critical for the assessment of subsequent field experiments undertaken in this thesis. Larvae were batch-tagged with the epi-fluorescent dye, calcein. Laboratory experiments demonstrated that the tagged larval shell was clearly visible in the spire of juvenile shells after >250 days. The recapture of tagged and released larvae from natural reefs, >500 days after release, confirmed the persistence of this tag. A reliable and cost-effective method for batch-tagging juveniles was through the use of a commercial diet that resulted in the distinctive blue-green colouration of the shell. The presence of this blue-green colouration differentiated released juveniles from those in naturally occurring populations, could be observed with the naked eye, without the need for a UV light source, and persisted on the spire of individuals for >900 days.

Methods of releasing *H. rubra* larvae and juveniles were developed and tested in a series of laboratory and field experiments. A deployment pump that included a pressurised container and hose, was used to successfully release larvae to natural reefs. The addition of GABA and refrigeration during simulated transport, and the stage of release from the deployment pump, significantly affected the number of larvae delivered through the pump. The release of larvae to physical shelters on the reef significantly increased the numbers that settled, and their survivorship. There was added complexity in the process of releasing juveniles than with that for larvae. The use of a deployment device (PVC tube, ~300 x 125 x 65 mm), that was securely placed onto the substratum, was integral to the successful release of juveniles. Use of these devices in a standard release protocol ensured the limited physical handling of juveniles and provided a simple, cost effective and efficient method for the release of large numbers to areas of natural reef.

A monitoring strategy was developed and tested to enable accurate estimates of the abundance and therefore survival of released *H. rubra* ,of a variety of life history stages, to be measured. The abundance of *H. rubra* juveniles is difficult to accurately assess on natural reef because of their cryptic distribution among complex topography in rocky habitat. As a consequence, the precision and relative accuracy of methods to sample released abalone was examined in a series of field experiments, and included the dispersal of juveniles from deployment devices. The most accurate and precise estimates of the number of *H. rubra* surviving were detected using methods that disturbed the habitat, i.e. turning over boulders, within a release location, and multiple sampling approaches were required to measure the abundance of abalone of different sizes. For larvae, the collection of boulders provided more accurate estimates of abundance than samples taken using a venturi-lift. For juveniles, thorough searching of boulder substratum and more replicates provided more accurate estimates of abundance. Further, stratified sampling among

habitats after natural disturbance revealed greater densities of *H. rubra* in 'solid habitat', and spatially stratified sampling indicated juveniles can disperse up to 10 m from their release point in <8 days.

The success of a stock enhancement strategy is determined by the net value it adds to a population. This necessitates estimates of the long-term survival and growth of released individuals, the impact released individuals have on the extant population, and the bio-economic feasibility of an enhancement strategy. Rates of survival and growth varied substantially among releases and locations. However, the long-term survival of batch-tagged and released larvae and juveniles demonstrated that local populations of abalone could be enhanced with significantly greater numbers of juveniles surviving at multiple release locations than at control locations after >2 years. The release of juveniles had no detectable affect on the mean total number of wild conspecifics or wild recruits over these time scales. Long-term survival of released larvae and juveniles was generally low (<0.03%, range: 0 - 0.03% and; <4%, range: 0 - 10%, respectively). However, at three of the twelve juvenile release locations it exceeded that expected for wild abalone (range: 4 - 10%). Growth rates of released juveniles (range: 18 - 47 $mm.yr^{-1}$) indicated that they would generally reach sexual maturity within 2 - 3 years and exceed the minimum legal length within 4.5 years. A deterministic, bio-economic model was used to analyse the net present value (NPV) of a stock enhancement strategy for *H. rubra* in NSW, against an alternate investment return of 10% p.a.. Positive NPV occurred where long-term survival was >4% and where growth rates were higher than average rates reported in this research, or beach price exceeded \$AUD $34.kg^{-1}$.

Low rates of long-term survival of larvae suggests their large-scale release is unlikely to provide a viable stand alone option to successfully enhance local populations of *H. rubra*. The survival and growth of released juveniles, to replicate locations, demonstrated that depleted local populations of *H. rubra* in NSW can be enhanced, and that a stock enhancement program can complement the NSW Abalone Fishery management strategy. The success of any large-scale stock enhancement program will be determined by the definition of its objectives. Outcomes from this thesis suggest that the greatest value of a stock enhancement strategy will be gained where its primary objective is to rebuild depleted populations, rather than optimise commercial yield through the release of individuals to overcome recruitment limitation or to harvest released individuals at a larger size. The decision to implement such a program, including explicit description of its primary objective, is required to be made among well-informed representatives of all stakeholders. Further, this decision needs to be made with a thorough understanding of the current biological structure of the populations, including the nature of population depletions, the economic status of the fishery and in light of current, complementary and alternative management arrangements that may provide comparative increases in net value.

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Life history stage definitions as used in this thesis

larvae:	post-fertilised egg through to settlement stage, developed larvae
settled larvae:	larvae attached to the substratum, having undergone metamorphoses or not, up to ~4 days after initial settlement; ~0.3 < 0.5 mm
early juvenile:	abalone ~5 - 60 days post-settlement; ~0.5 < 2 mm SL (shell length)
juvenile:	an abalone >60 days post-settlement and up to size at maturity; 2 < 90 mm SL
early adult:	a mature abalone within approximately the first year of maturity; 90 < 115 mm
adult:	a mature abalone after approximately the first year of maturity and of minimum legal length (MLL) in NSW, Australia (≥115 mm SL; prior to July 2008), unless otherwise stated (see bio-economic modelling, Chapter 6, where MLL ≥ 117 mm, as of July 2008)

General terms

Barrens habitat:	areas of reef dominated by crustose coralline algae and relatively high abundances of sea urchins (Underwood *et al.* 1991).
boulder field:	relatively loose boulders (250 - 600 mm diameter), that can be moved by a diver underwater, interspersed between areas of solid habitat within Fringe habitat, and not dominated by articulated coralline alga and other turfing alga, or containing large quantities of sediment among boulders)
deployment device:	also described in the text as a 'device'. Physical structure made of poly-vinyl-chloride (PVC) used to hold and release juvenile abalone (dimensions ~300 x 125 x 65 mm), unless otherwise stated
Fringe habitat:	the variety of subtidal reef habitats defined in (Underwood *et al.* 1991)
larval age:	age of larvae in days post-fertilisation (used in relation to hatchery-reared abalone larvae in flow through, filtered, UV sterilized seawater at 18^{o}C)
metamorphosis:	the irreversible attachment of a larvae to a substratum, identified by the presence of peristomal shell growth

peristomal shell:	shell deposited after metamorphosis
settlement:	the attachment of larvae to a substrate whether having undergone metamorphosis or not
shell length (SL):	maximum diameter of the shell (mm)
solid habitat	area of reef consisting of boulders cemented together, immovable boulders and patches of solid bedrock containing cracks and fissures
stock enhancement:	the means of adding individuals to a natural reef to augment the total number of individuals persisting through time to support a sustainable population, harvestable stock or both
survival:	the total number of abalone estimated to be within a release location (abundance) divided by the number of abalone released, expressed as a percentage, unless otherwise stated

1. GENERAL INTRODUCTION

1.1. POPULATIONS AND LIVING MARINE RESOURCE MANAGEMENT

The total number of individuals of a species living among a total number of spatially explicit areas can constitute a natural population (Andrewartha & Birch 1984). Similarly the number of individuals of a species living within one or other of these areas, defined within a biologically relevant spatial scale, can constitute a local population (see Andrewartha & Birch 1984, Berryman 2002, Camus & Lima 2002, and Kritzer & Sale 2004 for further discussion on defined spatial boundaries). Moreover, the number of individuals living within a defined space changes through time, with various natural processes, biological and environmental and their interactions controlling the amplitude and frequency of these changes.

Anthropogenic processes as well as biological and environmental factors affect populations (Plate 1.1). For example, the impacts of fishing on target and non-target species of marine animals and habitats has resulted in the ecological extinction of many species, the loss of local populations, and significant declines in the number of individuals in populations (Myers *et al.* 1997, Roberts 1997, Orensanz *et al.* 1998, Jackson *et al.* 2001, Christensen *et al.* 2003, Dulvy *et al.* 2003). In addition to direct anthropogenic processes resulting in declines of individuals and the loss of local populations, other environmental factors, hypothesised to be closely associated with anthropogenic influence, including outbreaks of disease (Harvell *et al.* 1999) and increasing seawater temperature, associated with patterns of global climate change (Harvell *et al.* 2002, Lackey 2003), can have significant effects on the size and stability of populations. Moreover, combinations of direct and indirect anthropogenic and natural processes can have significant and defined consequences on the viability of a population (Larkin 1996, Raimondi *et al.* 2002), particularly where that population may be small and biogeographically isolated and where demographic characteristics of the population may result in depensatory processes affecting the future population size (Fowler & Baker 1991, Myers *et al.* 1995, Babcock & Keesing 1999, Gardmark *et al.* 2003).

The demand for natural resources has led to their exploitation for economic and social benefit with fluctuations in demand often uncoupled from concerns for its sustainable use (Croxall & Nicol 2004). The management of natural resources, to ensure their sustainability and more recently to ensure the sustainability of the greater system in which they function, is commonly the ultimate responsibility of a publicly responsible regulatory agency. The dilemma facing natural resource managers is the development and implementation of a management strategy that allows for economic and social benefit to be gained from the utilisation of the resource

whilst ensuring its sustainability and biological relevance in the broader ecological system in which it functions.

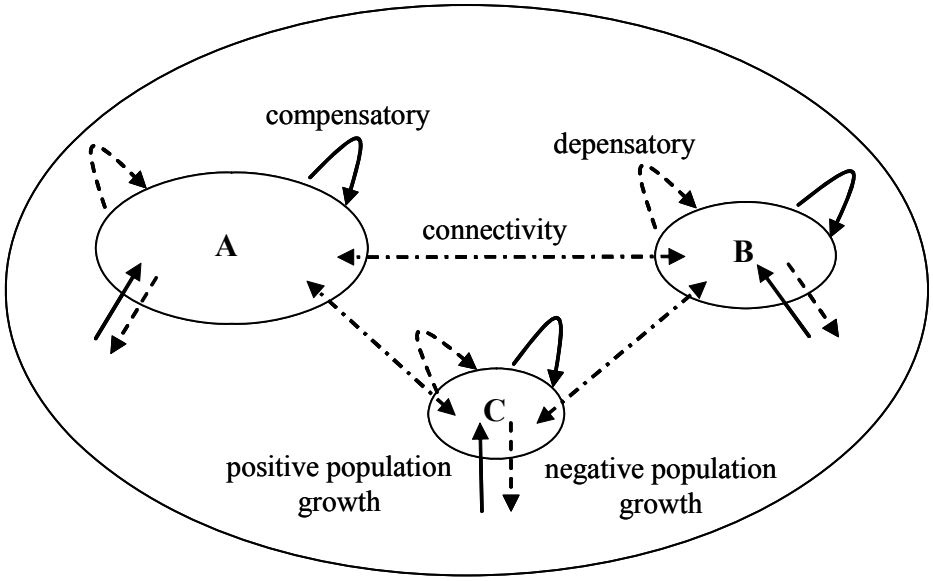


Plate 1.1 Local populations A, B and C, with natural and anthropogenic processes affecting functional population size through varying degrees of connectivity (from none to complete), demographic compensatory and depensatory mechanisms (e.g. density-dependent, growth, mortality and fertilisation success) and positive (e.g. recruitment, growth, emigration and successful management) and negative (e.g. natural mortality, immigration, disease and unsuccessful management (such as recruitment over-fishing)) processes affecting population growth.

The determination and understanding of changes in the demography of a population and of the biological and environmental processes that effect the abundance and distribution of individuals provide significant problems for resource managers. The fundamental concern being the inability to accurately predict natural changes in the demography of a population and the effects these changes have on the broader ecological system. Without the ability to accurately predict natural change it is impossible to accurately determine what effects direct anthropogenic processes, such as fishing, have and it is therefore increasingly difficult to develop and implement strategies to successfully manage the effects of these processes.

Traditional means of managing the effects of fisheries on populations has been through the spatial and temporal regulation of output (e.g. catch limits and size restrictions on target species) and input (e.g. effort limitation and gear regulation) controls. The introduction and enforcement of these controls traditionally attempts to at least maintain the stability of a presumed indicator of population size. Measures that have been and are accepted, and used as indicators of the status of exploited marine populations, include fishery-dependent measures such as catch, effort and catch-per-unit-effort and fishery-independent measures of the status of

the target species. The exclusive use of fishery-dependent measures to describe patterns of abundance of commercially fished stocks is not favoured (Hilborn & Walters 1992) but are often used because of the paucity of other measures. However, where supplementary measures are absent, the use of fishery-dependent measures are of greatest utility when interpreted in combination with demographic and biological characteristics of the target species.

The traditional response of a fisheries management agency to a perceived or real decline in a fishery is usually a further restriction in access to the resource with the underlying premise that a reduction in the number of individuals removed from the population will support its recovery. However, there are many examples where this response has not resulted in the recovery of the target population, either within a timeframe acceptable to the management agency and/or resource stakeholders, or at all (Tegner *et al.* 1996, Myers *et al.* 1997). This is particularly likely to be the case where management controls only have a partial influence. For example, where there are impacts from illegal fishing (Tegner 1993) or disease (Harvell *et al.* 1999, Raimondi *et al.* 2002). Large scale phenomena such as climate change may also cause negative or positive synergies on a population that is already under pressure (Harvell *et al.* 1999, Harvell *et al.* 2002). Moreover, unclear communication and inaccurate interpretation of information from resource scientists to managers, political will to respond to uncertain, but negative forecasts regarding the status of marine resources and delay in the implementation of management strategies has lead to the dramatic decline of marine populations and the loss of economically valuable resources (Hutchings *et al.* 1997, Lauck *et al.* 1998, Orensanz *et al.* 1998, Rosenberg 2003).

In light of obvious failures of traditional management strategies among many fisheries of the world (Hilborn & Walters 1992, Orensanz *et al.* 1998, Hilborn *et al.* 2003, Lackey 2003), it has become clear that the interpretation of predicted change to natural populations in response to natural and anthropogenic processes require the adoption of principles of precaution (FAO 1996, Foster *et al.* 2000, Kriwoken *et al.* 2001). There is the understanding that, by adopting these principles, decision makers err on the side of caution when interpreting uncertain scientific advice for setting exploitation levels for natural resources. Moreover, the application of these principles into management frameworks has resulted in the reassignment of the onus of proof from that of a management agency having to demonstrate that a process detrimentally affects a population or habitat to that of the benefactor demonstrating otherwise (Dayton 1998, Kriwoken *et al.* 2001).

In addition to adopting these principles of precaution, major shifts in the development of strategies to manage natural marine resources, within the last decade, have been to acknowledge the lack of understanding surrounding traditional single species fisheries operating in complex ecological systems. This has led toward the provision of improved risk assessments and

decision analyses into methods used to estimate population size and predict population change (Hilborn & Walters 1992, Hilborn 2003). A fundamental shift in more recent resource management strategies has been to include non-impacted components of ecological systems, i.e. marine reserves, into the management framework (Lauck *et al.* 1998, Conover *et al.* 2000, Lubchenco *et al.* 2003). The premise of adopting marine reserves being that, even if precautionary traditional management strategies fail to adequately protect a population or habitat from controllable activities operating within the complex ecological system, the provision of a non-impacted area or areas will, at least potentially, allow for the proliferation and reestablishment of the population or habitat back into unprotected areas.

Specific benefits of marine reserves to the security of harvested marine populations, non-target populations, marine habitats and in fact those trying to manage these resources have been widely published (Lubchenco *et al.* 2003). However, not all species benefit equally from the restriction of regulated activities, including fishing, afforded by the implementation of marine reserves (Barrett *et al.* 2007, Barrett *et al.* 2009) and the adoption of principles of precaution. In fact, populations with demographic characteristics that demonstrate depensatory mechanisms and that can provide significant reward through illegal harvest e.g. haliotids, are unlikely to benefit from the implementation of areas closed to and protected from regulated activities, including fishing, without the implementation of effective enforcement (Tegner *et al.* 1992, Tegner 1993, Tegner *et al.* 1996, Russ 2002). Further, the implementation of areas protecting all species from regulated activity may in fact have unexpected and negative effects on some population sizes. For example, the abundance of *Haliotis rubra* in Tasmanian waters unexpectedly decreased in protected areas where they were exposed to an increase in abundance of predators (Barrett *et al.* 2009). Cases such as this indicate that alternate management strategies are likely to be required in concert with enforced traditional strategies to address declining numbers of individuals within a population, or the loss of local populations.

1.2. MARINE STOCK ENHANCEMENT

The term stock enhancement has generally been used to describe a means of supporting the recovery or expansion of a local population for the purpose of re-establishing that population, rebuilding a population that has declined to the point of being unable to support a viable fishery or to supplement a population to support a recapture fishery (Blankenship & Leber 1995, Travis *et al.* 1998). Stock enhancement is a strategy that can compliment more traditional management options to help address declining numbers of individuals or the loss of local populations. More recent terminology has developed to more precisely reflect specific objectives of a management strategy supporting the supplement of wild stocks. The terms 'restocking' and 'stock enhancement' have been defined to reflect objectives of rebuilding severely depleted stocks to

support viable fishing practices and increasing productivity of existing fisheries, respectively (Bell *et al.* 2005, Bell *et al.* 2008). For the purpose of this thesis the term stock enhancement is used generically to describe the release of hatchery-reared individuals to increase the abundance of the target species, irrespective of the ultimate objective of any possible future management strategy. The implication being that the utility of a stock enhancement program can be resolved and the subsequent success or failure of its implementation determined through the specific objectives of its associated management strategy, be that 'restocking' or 'stock enhancement', as defined by Bell *et al.* (2005, 2008). A stock enhancement strategy may take the form of one or a combination of programs including habitat regeneration, the relocation of wild stock or the rearing and release of larvae or juveniles. The adoption of one or a combination of these methods would be dependent upon the objectives of the strategy and the specific circumstances of the resource to which it is being applied.

The general aim of this thesis was to determine the utility of releasing larvae and juveniles for the stock enhancement of blacklip abalone (*H. rubra*) in New South Wales, Australia. Stock enhancement has been used as a fisheries management tool to supplement existing wild populations to support catches in fisheries in many marine environments throughout the world (Hilborn 1998, Masuda & Tsukamoto 1998, Chiu Liao 1999, Hilborn 1999, Kitada 1999, Bell *et al.* 2005). Many of these stock enhancement programs have been reviewed and their continuation criticised, primarily because of a lack of scientific evidence of their contribution to the targeted population (Hilborn 1998, Welcomme 1998). However, many of these programs undoubtedly contribute individuals to the fishable stock and support extensive fishing industries (Chiu Liao 1999, Kitada 1999, Simizu & Uchino 2004, Bell *et al.* 2005). The acquisition of evidence to support a hypothesis of significantly adding individuals to a population is made difficult in that any contribution made is often masked by a background of natural variability in recruitment. This is particularly the case where released individuals are not identifiable (Hilborn 1998). In addition, demonstrable evidence of significantly enhancing a population is made more achievable where that population does not operate as a single stock (Hilborn 1998, Doherty 1999), i.e. local population demography is not significantly affected by the larger population, where the population is relatively sedentary and where the enhancement effort is aimed towards largely depleted populations (Hilborn 1998).

1.2.1. Abalone fisheries and stock enhancement

World fisheries are under increasing international and domestic demand (Jackson *et al.* 2001, Christensen *et al.* 2003). This increasing demand for resources and the inability to accurately predict the responses of populations to natural and anthropogenic processes, and manage them through appropriate management strategies has lead to the loss of economically and socially

valuable fishing industries (Myers *et al.* 1997), including abalone industries (Tegner *et al.* 1992, Hobday *et al.* 2001). With persistent and growing global demand for abalone (Gordon & Cook 2001, 2004, FAO 2010) the current status of many abalone stocks throughout many of the worlds abalone fisheries indicate reductions in either fishery-dependant indices, such as catch, effort or catch-per-unit-effort or combinations thereof (Guzman del Proo 1992, McCormick *et al.* 1994, Rudd 1994, Masuda & Tsukamoto 1998, Tarr 2000, Shepherd & Rodda 2001, Worthington *et al.* 2001, Breen & Kim 2003, Tarbath *et al.* 2005) or reductions in fishery-independent indices of the status of these resources (Worthington *et al.* 1999, Hobday *et al.* 2001, Worthington *et al.* 2001, Breen & Kim 2003).

The efficacy of enhancing populations of abalone through the release of hatchery-reared larvae or juveniles to areas of natural reef has been investigated at different scales for a number of abalone fisheries throughout the world (Tong *et al.* 1987, Schiel 1993, McCormick *et al.* 1994, Kojima 1995, Preece *et al.* 1997, Masuda & Tsukamoto 1998, Rogers-Bennett & Pearse 1998, Cook & Sweijd 1999, Shepherd *et al.* 2000, Gutierrez-Gonzalez & Perez-Enriquez 2005). Within many abalone stock enhancement programs the means of successfully releasing individuals to maximise their survival and the subsequent means of assessing that survival are problematic. It has been well documented that a number of processes affect the settlement, metamorphosis and growth of hatchery-reared larvae (McShane 1992, 1995, Roberts 2001, Daume *et al.* 2004). However, much of the research in this area has focused on investigating cues for stages of settlement and identifying appropriate substratum and conditions to maximise larval settlement and survival primarily for the benefit of commercial abalone hatcheries. In addition, a variety of processes that affect the short (<7 days) and medium (<3 months) term survival of released abalone, extrinsic to processes affecting mortality in wild populations, have been identified. For example, processes involved in the handling and transportation of individuals can significantly affect their health prior to release, which subsequently increases rates of mortality soon after release (Schiel 1992, 1993, McCormick *et al.* 1994, Shepherd *et al.* 2000). Some studies indicate that rates of mortality after release are similar between wild and hatchery-reared juvenile abalone (Schiel 1992, McCormick *et al.* 1994). Because of the cryptic habitat preferred by juvenile abalone, it is often difficult to obtain representative and reliable samples of this life history stage, including those introduced to wild populations. Various methods for sampling these types of habitat have been described, involving varying degrees of disturbance to the substratum, each with its own bias and limitations (Prince & Ford 1985, Shepherd & Turner 1985, McShane & Smith 1988). Despite difficulties in releasing and sampling larvae and juveniles, various stock enhancement programs for abalone have indicated the potential for successfully enhancing depleted populations (Ebert & Ebert 1991, Schiel 1992,

1993, Sweijd *et al.* 1998) and in fact a number of Japanese stock enhancement programs support commercial fisheries (Kojima 1995, Masuda & Tsukamoto 1998, Simizu & Uchino 2004).

1.3. BIOLOGY AND ECOLOGY OF ABALONE

Abalone are demersal, relatively sedentary, dioecious, broadcast spawning molluscs, of the family Haliotidae. Spawning periodicity is protracted in many species (Shepherd & Laws 1974) and may not be an annual event (Sainsbury 1982), with limited literature suggesting some synchronicity to spawning among individuals within a population (Shepherd 1986b, Stekoll & Shirley 1993). Fertilisation success has been demonstrated to be a function of distance between spawning individuals, with successful rates of fertilisation dropping to below 5% over distances of greater than two metres (Babcock & Keesing 1999). Fertilised eggs hatch after approximately 24 hours and larvae are lecithotrophic, developing in the plankton for periods of time from several days to over a week, depending on various factors including water temperature (Leighton 1972). Larval settlement is strongly associated with non-geniculate (crustose) coralline alga (Shepherd & Daume 1996), although successful settlement and metamorphosis is not restricted to the surfaces of these alga (Roberts 2001, Huggett *et al.* 2005). Moreover, despite fertilisation occurring in the water column and larvae developing in the plankton there is evidence to suggest that the dispersal of larvae from parent stock may only range from metres, in species including *H. rubra* (Prince *et al.* 1987, McShane *et al.* 1988a, Prince *et al.* 1988a, McShane 1992), to 100s of metres and even kilometres in other species (Wells & Keesing 1990, Shepherd *et al.* 1992, Tegner 1992, Sasaki & Shepherd 2001). Small juveniles (~2-20 mm) are cryptic in the wild (Shepherd & Turner 1985, McShane 1992), but have higher rates of mortality than larger juveniles and adults (Shepherd & Breen 1992, Shepherd 1998). Larger juveniles and adults inhabit subtidal rocky reefs, with *H. rubra* more commonly being found in aggregations primarily under boulders or in cracks and fissures in the bedrock (see Plate 1.2 for general life history stages as described in this thesis).

H. rubra are relatively sedentary, aggregated, highly valued individuals with a protracted spawning period and limited capabilities of larval dispersal. It is therefore unlikely that they can re-establish themselves quickly in local populations after a major impact affecting population size. Ironically, many of the demographic and economically favourable characteristics of *H. rubra* that make it a particularly suitable species for stock enhancement are also characteristics that make it vulnerable to continued population decline and protracted recovery after significant events of disease or over-fishing.

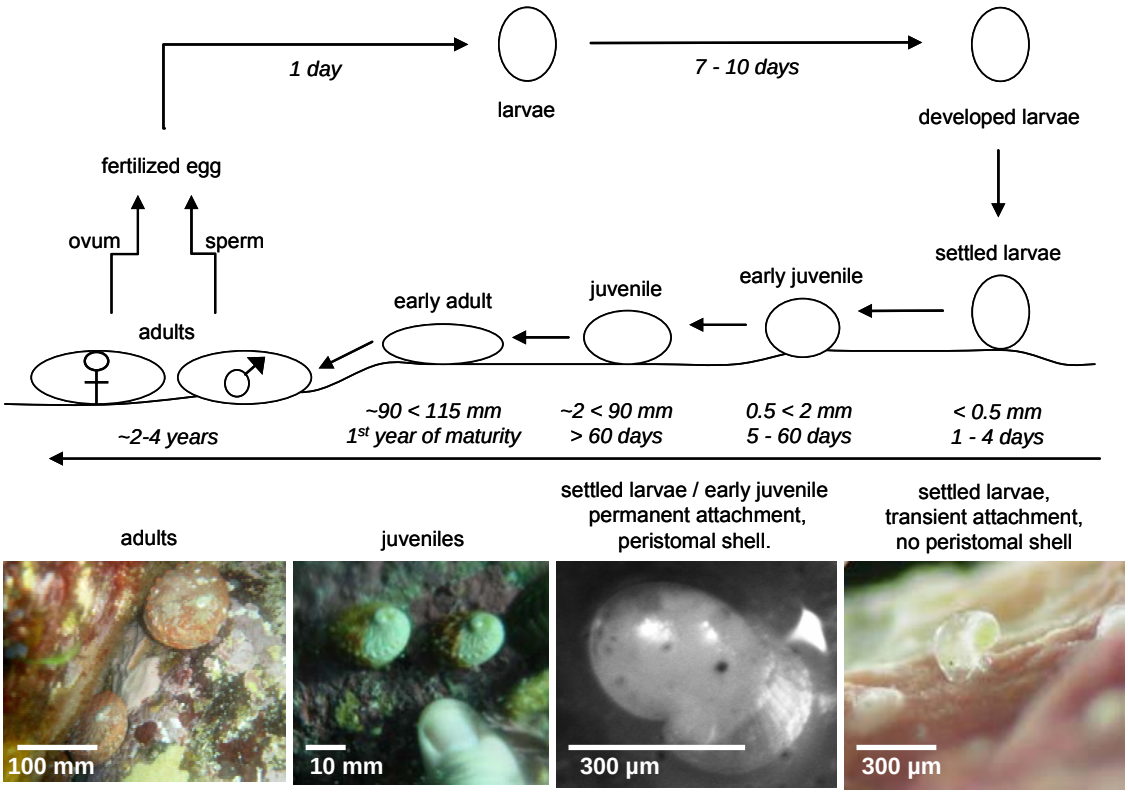


Plate 1.2 Life history stages of *H. rubra* as described in this thesis, with indicative size range, timeframes and photographs of indicative forms. Photographs taken by the author, with the exception of "settled larvae" which is *H. iris*, modified from Naylor *et al.* (2006b).

1.4. NEW SOUTH WALES ABALONE FISHERY

The abalone fishery in New South Wales (NSW) is markedly different from other abalone fisheries, particularly in Australia, in that it is a single species (*H. rubra*), recruitment driven fishery (Andrew *et al.* 1997). The abalone resource in NSW is most abundant in the southern regions of the state with a patchy distribution of aggregations of tens to hundreds of individuals commonly separated by distances from tens of metres up to kilometres, extending north, with few aggregations found north of Coffs Harbour (30°39.00' S; Andrew *et al.* 1996).

Indicators of the status of the NSW abalone fishery during the late 1990s suggest that it was fully exploited, with populations of abalone within the central areas of the state, between Port Stephens and Jervis Bay, having undergone collapse (Anon 2005c). Fishery-independent surveys of relative abundance, at fixed locations over a number of years, indicate that some of these abalone populations have declined to less than 5% of their previous levels within 5 years (Daly 2004). These declines have been attributed primarily to the infection and subsequent mortality of individuals by a protistan parasite, likely to be *Perkinsus olseni* (Daly 2004). Moreover, some local populations of abalone, including those beyond those regions affected by disease, have declined to less than 35% of previous levels within 1 year (Worthington *et al.*

1999). These levels of decline are most likely a result of illegal fishing, as indicated by the absence of abalone greater than ~65 mm in length, in contrast to populations exhibiting more representative non-cryptic size structures below the minimum legal length. In addition, there are concerns that previous increases in the distribution of the sea urchin *Centrostephanus rodgersi* has resulted in areas of reef being maintained relatively free of macro-algae and being unable to support previous populations of abalone. The removal of urchins from areas of reef has been demonstrated to promote the regeneration of habitat able to support abalone populations (Andrew *et al.* 1998, Worthington & Blount 2003) and through the introduction of abalone, has resulted in the long-term persistence of these populations (Andrew *et al.* 1998).

Despite forecasts of increased pressure being placed on remaining populations of abalone in NSW (Worthington *et al.* 2001, Anon 2005c), there are significant areas of reef where populations of abalone have supported >75% of the historical catch in the commercial fishery and still support significant commercial, recreational and indigenous fisheries. In addition, there are substantial areas of reef where abalone were recently abundant and where habitat seems appropriate to support future populations. The significant declines of abalone populations on reefs within their northern distribution does however provide serious grounds for concern for the sustainable harvest of abalone in NSW (Anon 2005c). The levels of depletion of some local populations have not been previously documented in the history of this resource. Further, after up to 10 years of decline in the abundance of abalone in these wild populations there has been little recovery despite the closure of many of these areas to regulated fishing (Anon 2005c).

1.5. RESEARCH IN THIS THESIS

The demographic and economic characteristics of *H. rubra* make it a good candidate for stock enhancement. Further, the depletion of local populations, combined with little indication of their recovery after substantial changes in traditional management controls, suggest that a complementary stock enhancement strategy may support the persistence and growth of these populations. The principle objective of this research was to provide the methodology, baseline targets and recommendations for the implementation of a stock enhancement management strategy for local populations of blacklip abalone in NSW, Australia.

The research in this thesis consists of a series of interrelated laboratory and field based experiments structured to address the objectives (Section 1.5.1). The timescales over which experiments were done ranged from 1 day to >2 years (Table 1.1). Much of the emphasis of the work done in chapters 2-5 is focused on the period of time within ~60 days post-settlement and post-release of *H. rubra* larvae and juveniles, respectively. It is generally within this timeframe that maximum rates of mortality operate (Figure 1.1). The potential to reduce this early mortality, through the development of procedures to handle and release individuals from a

hatchery to the field, can substantially affect rates of long-term survival (>2 years; Chapter 6). Processes involved in the handling and transport of larvae and conditions observed at release locations were tested in the laboratory to determine their effects on the settlement, metamorphosis and growth of larvae (Chapter 2). Outcomes from these experiments contributed to the development of a standard release protocol for larvae. Methods to indelibly batch-tag larvae and juveniles were developed, tested and routinely applied to all abalone released (Chapter 3). Batch-tagging enabled released individuals to be positively identified when recaptured. Methods to successfully release larval and juvenile abalone were investigated in laboratory and short-term field experiments (Chapter 4). Further, field experiments were done to develop a monitoring strategy to assess the long-term survival and growth of released larvae and juveniles (Chapter 5). The utility of releasing *H. rubra* to enhance local conspecific populations was investigated through a series of controlled field release experiments. Estimates of long-term survival and growth were integrated into a deterministic bio-economic model that considered the net present value of a simulated stock enhancement strategy. Outcomes from these investigations were interpreted to provide recommendations for the implementation of a strategy to enhance local populations of *H. rubra*, in NSW, Australia (Chapter 6).

1.5.1. Objectives

The objectives of this thesis were to:

1. Investigate the effects of (i) factors related to handling and transport; and (ii) conditions imposed following release, on the settlement, metamorphosis and early growth of *H. rubra,* to develop a protocol for their handling, transport and release;
2. Develop and test methods that enabled batches of larval and juvenile *H. rubra* to be reliably and consistently tagged, and subsequently identified on natural reefs over periods of time ranging from 1 day to >2 years;
3. Develop and test methods to enable *H. rubra* larvae and juveniles to be released to natural reefs, allowing their settlement and survival;
4. Develop a monitoring strategy to accurately estimate the abundance of released *H. rubra* within release locations;
5. Quantify the long-term (2 year) survival and growth of batch-tagged *H. rubra* released to natural reefs to provide minimum targets for a stock enhancement management strategy for the NSW abalone fishery;
6. Determine the impact of releasing juveniles on the abundance of wild *H. rubra* populations;
7. Provide a bio-economic analysis of a stock enhancement strategy for *H. rubra* in NSW; and
8. Provide recommendations for the implementation of a stock enhancement management strategy for local populations of blacklip abalone in NSW, Australia.

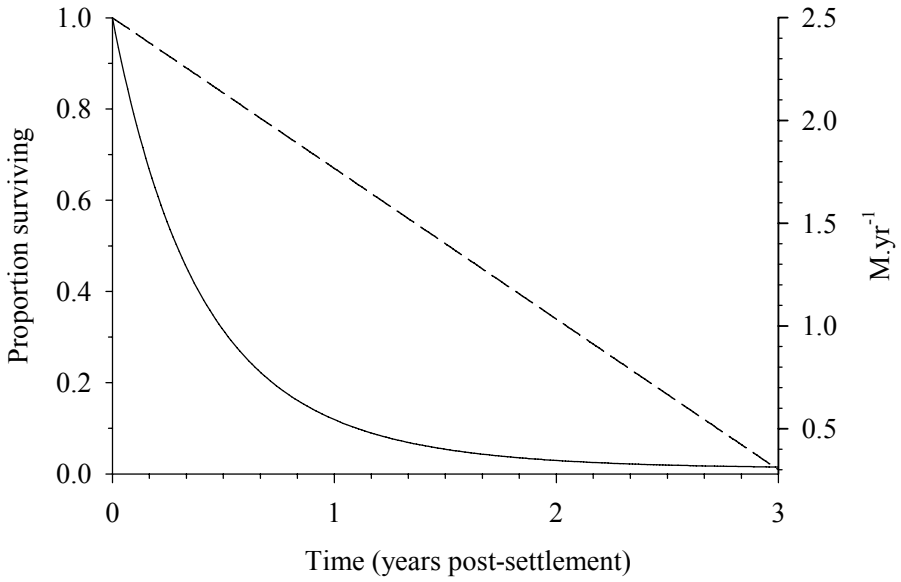
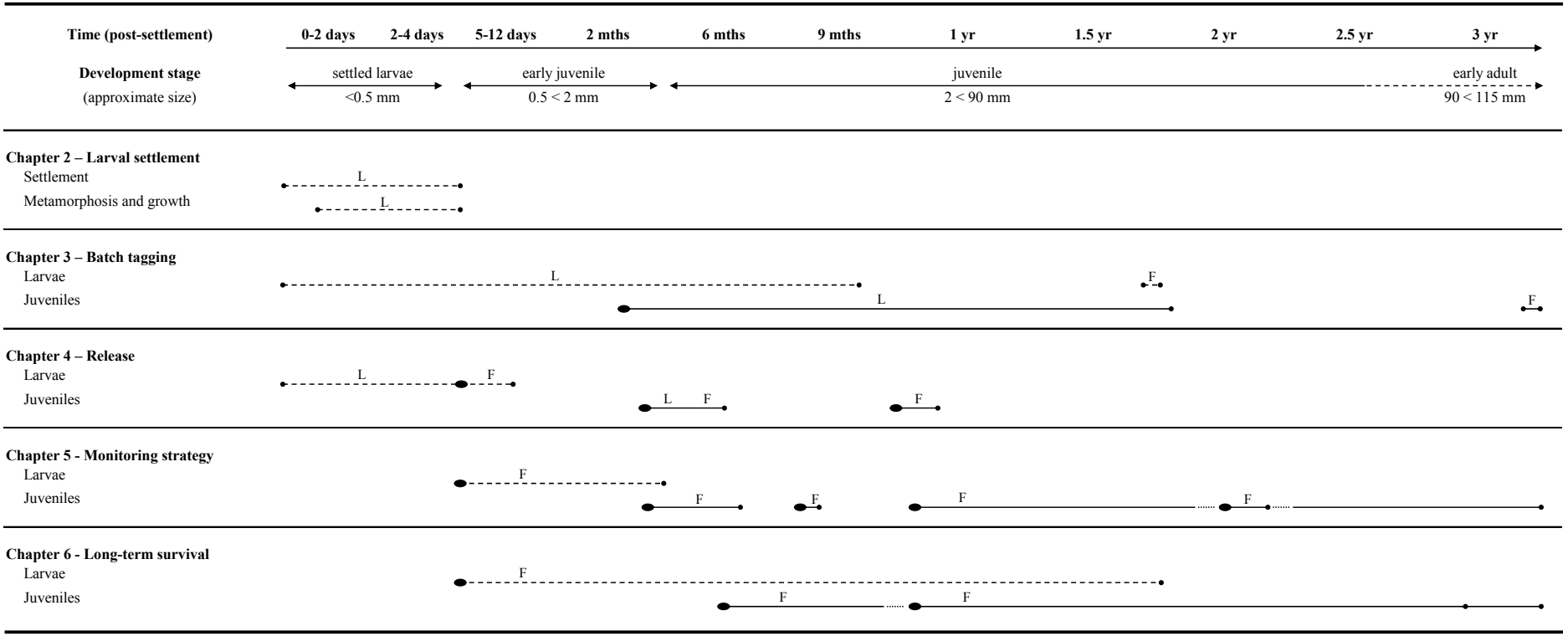


Figure 1.1 The proportion of abalone surviving through time, from 0 to 3 years (solid curved line), generated from a linear decline in instantaneous rates of natural mortality ($M.yr^{-1}$) from 2.5 to 0.3 (dashed straight line), as suggested by Shepherd and Breen (1992) for wild *H. rubra*.

Table 1.1 General overview of laboratory (L) and field (F) experiments done throughout Chapters 2 - 6 in this thesis, indicating the use of larvae (dashed lines) or juveniles (solid lines), the points of release (large black circles) at indicative sizes and ages (Time, post-settlement) and the period of time over which experiments in each chapter were done (small black circles, end point). Note: not all experiments are represented by independent lines and points of release as some experiments used individuals of similar size and were sampled for similar periods of time, as such, lines and release points should be viewed as indicative of the experiments done within each chapter.



2. FACTORS AFFECTING THE SETTLEMENT OF *HALIOTIS RUBRA* LARVAE

2.1. INTRODUCTION

Abalone are dioecious broadcast spawners that produce planktonic larvae. These larvae are lecithotrophic, although there is evidence that they acquire additional nutritional input from dissolved organic matter from their surrounds (Manahan & Jaeckle 1992). Fertilised eggs commonly take between 4-15 days to develop into larvae that can successfully settle, i.e. attach to a substrata, undergo metamorphosis and grow (Hahn 1989b). The period of time prior to successful settlement may be quite protracted and can be significantly affected by a number of processes (see reviews by McShane 1992, Morse 1992, McShane 1995, Roberts 2001). However, increases in the time spent in the water column or ranging between substrata can significantly affect the rate of successful settlement and subsequent growth (Moss & Tong 1992b, Roberts & Lapworth 2001).

The successful settlement of abalone is a process consisting of a number of different, potentially reversible stages (Roberts 2001). The first stage, settlement or attachment, consists simply of the larvae settling out of the water column and adhering to a suitable substrata. If larvae have not had a protracted time in the water column or there are not appropriate cues, they may detach and resume the planktonic phase. Following settlement, larvae undergo metamorphosis, where they develop a mouth and are committed to the substratum; this process is irreversible. Following metamorphosis larvae begin to feed and start depositing their post-larval, peristomal shell.

Most research investigating the settlement of abalone larvae has been for the benefit of aquaculture, with a focus on identifying and quantifying conditions affecting their settlement, metamorphosis and growth, including the effects of gamma-aminobutyric acid (GABA) (see reviews by McShane 1992, Morse 1992, McShane 1995, Kawamura 1996, Roberts 2001). An inclusive subset of these investigations has focused on the effect that extracts, physical growth forms and different species of crustose coralline algae (CCA), other algae and diatom films have on the settlement, metamorphosis and growth of abalone larvae (Morse & Morse 1984, Moss & Tong 1992a, Shepherd & Daume 1996, Roberts & Nicholson 1997, Daume *et al.* 1999, Daume *et al.* 2000, Roberts *et al.* 2004, Huggett *et al.* 2005). Application of the results has provided opportunities for improved efficiencies in commercial aquaculture and supported hypotheses regarding the processes affecting patterns of natural recruitment of abalone (McShane 1992, 1995, Roberts 2001, Huggett *et al.* 2005).

There are few data on the response of abalone larvae to conditions they are exposed to in the field at, or soon after, settlement (but see Shepherd & Turner 1985, McShane & Naylor 1995b,

Shepherd & Daume 1996, Naylor & McShane 2001, Sasaki & Shepherd 2001), although the association of settled abalone larvae with CCA has been well documented (Shepherd & Turner 1985, Shepherd & Daume 1996, Day & Branch 2000b). Similarly, although methods of moving abalone larvae from a hatchery to the field for release have been described (Tegner *et al.* 1986, Tong *et al.* 1987, Schiel 1992, Preece *et al.* 1997), and the time taken by larvae to settle has been proposed to affect their successful settlement (Schiel 1992, Preece *et al.* 1997) and survival (McShane 1992, Shepherd *et al.* 1992, McShane 1995, Roberts & Lapworth 2001), little is known about settlement responses of abalone larvae following exposure to the range of conditions imposed during handling, transport and release from a hatchery to a field release location.

The effects of handling, transport and release conditions have potentially important implications for the successful settlement and growth of abalone (Beaudry 1983, Naylor & McShane 2001, Roberts & Lapworth 2001, Phillips & Shima 2006). As a consequence, these processes can either directly affect successful settlement and survival, or the likelihood of larvae to settle within the boundaries of a release location (Schiel 1992, Preece *et al.* 1997). Either consequence will effect future estimates of their successful settlement and survival and therefore the measurement of success of releasing larvae to enhance a local population.

The general aim of the work done in this chapter was to develop a standard protocol to handle, transport and improve potential settlement of larvae released to field locations to investigate their potential to enhance local populations. The specific aims of the experiments designed were to: 1) determine if a glass microscope slide conditioned in filtered seawater for ~4 weeks to develop a layer of benthic diatoms (*sensu* standard settlement substrata), could provide a comparable settlement response of larvae to natural substrata, and therefore support the use of standard settlement substrata in subsequent laboratory experiments; 2) determine if the use of gamma-aminobutyric acid (GABA) reduced the time larvae spent in the water column, to support the use of a standard concentration of GABA in field releases; 3) ensure that the effect of treatments directly related to processes of handling and transport of larvae for field releases, including refrigeration (i.e. a potentially simple and effective storage and transport method) and an abrupt temperature increase prior to settlement (not uncommonly experienced during the storage, transportation and preparation for release of larvae to a field location), did not significantly reduce the capability of larvae to successfully settle; and 4) identify processes affecting the successful settlement of larvae post-release, including the time larvae were exposed to a settlement substrata and the flow of water across a settlement substrata (simulating water movement in the field, common during the release of larvae), to support release methods to improve potential settlement within field release locations.

Several experimental designs consisted of combinations of factors and their treatments. In addition, some experiments were repeated with consistent treatments among different batches of larvae to investigate variation in the effects of treatments among batches. In discussion, conclusions from the results of this series of controlled laboratory experiments are drawn to describe how various processes affecting the successful settlement of *H. rubra* are likely to impact on efforts to enhance local populations of abalone and how these data inform the development of a standard protocol, essential for the release of larvae to natural reefs, as described in Chapters 4, 5 and 6.

2.2. MATERIALS AND METHODS

A series of 12 laboratory experiments were undertaken to test hypotheses regarding the consistency of the settlement response of larvae to treatments of factors relating to their handling and transport to field release locations, as well as post-release processes (Table 2.1). In addition, a subset of these experiments were used to investigate the effect levels of treatments of these factors had on the metamorphosis and early growth of settled larvae (Table 2.2). A standard experimental protocol was adhered to throughout each experiment (Section 2.2.5), including the use of a novel, miniature, down-welling system (Section 2.2.6). These results were used to support the development and use of a standard protocol to release larvae to natural reefs, described in Chapters 4, 5 and 6.

2.2.1. Settlement substratum

Experiments 1, 2 & 3

Observations from samples collected from pilot releases of larvae to field locations indicated that primary settlement substrata consisted of bedrock, or boulders covered with CCA. To reduce the likelihood of confounding laboratory results with different types of CCA collected on rocks from local reefs, and due to the logistic constraints of carrying out a series of experiments on CCA covered rocks collected regularly from local reefs, the subset of experiments using CCA substratum (Experiments 1-3, Table 2.1) were designed to test the hypothesis that the settlement response of larvae to standard settlement substratum was consistent with that to CCA coated rocks, in order to support the use of a standardised settlement substrata in subsequent experiments.

In experiment 1 it was also hypothesised that the proportion of settled larvae that metamorphosed, and their size after 96 hours on a standard settlement substrata, would not be significantly different with that on CCA covered rocks. Further, in experiments 2 and 3, it was hypothesised that the settlement responses of larvae to these substratum would be consistent

among treatments of the factor flow, and factors flow and refrigeration, respectively (described below). Results from these experiments, and the need to minimise variation in the settlement response of larvae to differences in the composition of the settlement substrata, supported the use of a standard settlement substrata in subsequent experiments.

In experiments 1-3 two types of settlement substratum were used. Substrata one (i.e. standard settlement substrata) consisted of a 75 x 25 mm glass microscope slide, carrying a bio-film of benthic diatoms. The bio-film on this substrata was created by conditioning the slides in aerated, filtered (10 μm), non-recirculated seawater for 4 to 6 weeks. Immediately prior to their use in each experiment the standard settlement substrata was gently wiped to remove any macroscopic filamentous algal growth, if present. Substrata two consisted of two small CCA coated rocks, with a combined displacement volume of approximately 70 ml. Rocks coated in CCA were collected from coastal reefs up to two days prior to use in the experiments and were held in aerated, filtered (10 μm), non-recirculated seawater.

2.2.2. Factors relating to handling and transport

2.2.2.1. Gamma aminobutyric acid

Experiment 4, 5, & 6

Experiments 4-6 (Table 2.1) were designed to test the hypothesis that the settlement response of larvae to a standard concentration of GABA (1 x 10^{-6}M) would be significantly greater to that of larvae exposed to a control (0 x 10^{-6}M).

In experiment 4 an additional concentration, i.e. 2 x 10^{-6}M, was included to test the hypothesis that the settlement response to GABA was concentration dependent. This was done as some literature describes the likelihood of a high concentration of GABA, such as 2 x 10^{-6} M, further reducing the time larvae spend in the water column (Morse 1992, Roberts 2001). However, this literature also cautions against possible high rates of larval mortality at these concentrations. Therefore, the proportion of settled larvae that metamorphosed and the size of settled larvae, after they had been on the settlement substrata for 24 and 48 hours, was measured. These data were used to test the hypothesis that the proportion of settled larvae that metamorphosed, and their size after 24 and 48 hours would not be significantly different among levels of GABA. Larvae were measured from a random selection of 4 of the available 8 replicates in this experiment (Table 2.2).

In experiments 5 and 6, it was also hypothesised that the settlement response of larvae to a control and standard concentration of GABA (1 x 10^{-6}M) was consistent among levels of the treatment flow, and after 24 hours of refrigeration, respectively (described below). Results from

experiments 1-3 were used to support the use of the standard concentration of GABA (1×10^{-6}M) in releases of larvae to field locations described in Chapters 4, 5 and 6.

GABA treatments were mixed to the required concentration in a 1 L container holding larvae and stirred for 15-20 minutes. Following the addition of GABA treated larvae to each replicate down-weller in experiment 4, water flow to each replicate was turned on after the first 24 hours. Different GABA treatments were assigned separate baths, to minimise the chances of cross contamination of concentrations among the different treatments.

2.2.2.2. Refrigeration

Experiments 3, 6, 7 & 8

Refrigerated storage of larvae was investigated in experiments 3 and 6-8 (Table 2.1) as a means of storing ready to settle larvae prior to release in the field. Three standard levels of refrigerated storage were investigated: 1) a control (ambient temperature 18^{o}C); 2) refrigeration (4-6^{o}C) for 24 and; 3) refrigeration (4-6^{o}C) for 48 hours. These durations were chosen because they reflected the approximate times required to transport and release larvae from the hatchery to field locations. Prior to treatments all larvae were reared to 7 days post-fertilization following the protocol describe below (Section 2.2.5).

Experiments 3, 7 and 8 were designed to test the hypothesis that the settlement response of larvae would not be significantly different between levels of refrigeration. Further, in experiments 3 and 8 it was also hypothesised that the settlement response of larvae to refrigeration for 0, 24 and 48 hours would be consistent among levels of treatments of the factors substrate and flow, respectively, and in experiment 8, that the proportion of settled larvae that metamorphosed, and their size would not be significantly different among levels of refrigeration and flow after exposure for 24 and 48 hours.

In experiments 3, 6, 7 and 8 refrigerated larvae were transferred to a 1 L container of filtered (10 μm) seawater, through which oxygen was bubbled for ~3 minutes prior to the container being sealed and placed in a domestic refrigerator for the required treatment time (24 or 48 hr.). Before larvae were placed in the down-wellers they were acclimated to ambient temperature over a period of approximately 3 hours. 'Control' larvae were maintained in a larval rearing system for the same period of time as the refrigerated storage treatment. In experiment 8 the proportion of settled larvae that metamorphosed, and the size of settled larvae after they had been on the settlement substrata for 24 and 48 hours, was measured (Table 2.2).

2.2.2.3. Temperature shock

Experiments 9 & 10

Three standard water temperature treatments (i.e. temperature shock) were investigated. These were: 1) a control (ambient temperature 18^{o}C); 2) a temperature increase of 5^{o}C; and 3) a temperature increase of 10^{o}C. These treatments were chosen to simulate the extreme range of temperature fluctuations either inadvertently imposed during the processes of handling and transportation of larvae (such as in the deployment pump – Section 4.2.1.1, immediately prior to release) or the potential temperature shock experienced at the time of release to field locations at depths of 5-10 m (i.e. often cooler than surface or ambient water). It was hypothesised that the settlement response of larvae would not be affected by variation in temperature (Experiments 9 and 10, Table 2.1). It was also hypothesised that the metamorphosis and size of settled larvae would not be affected by variation in temperature prior to settlement after 96 and 48 hours, in experiments 9 and 10 respectively, and in experiment 10, that this response would be consistent among the levels of the treatment flow.

To achieve the required temperature shock treatments, larvae in 1 L containers of seawater were transferred to water baths of 18, 25 or 32^{o}C, and held for ~30 minutes prior to exposure to the settlement substrata in the down-weller, at the ambient temperature of 18^{o}C. In experiments 9 and 10 the proportion of settled larvae that metamorphosed, and their size after 96 and 48 hours, respectively, and among levels of the factor flow in experiment 10, was measured (Table 2.2).

2.2.3. Factors imposed following release

2.2.3.1. Exposure time

All experiments

In each of the experiments, larvae were exposed to the settlement substrata for a range of times. Short periods of time (i.e. 30 and 60 seconds) were chosen to help determine if larvae could settle quickly and therefore be more likely to remain within a relatively small area for field releases. Longer periods of time (i.e. 24 and 48 hr.) were chosen to investigate the effects of treatments on the competency of larvae to successfully settle.

It was hypothesised that the settlement of larvae would be significantly affected by the time they were exposed to a settlement substrata and that the pattern of settlement would be consistent among other treatments. In experiments 4 and 8 the proportion of settled larvae that metamorphosed and the size of settled larvae was measured (Table 2.2). These data were used to test the hypotheses that the proportion of settled larvae that metamorphosed, and the size of settled larvae was significantly different among levels of the treatment time and that these

patterns were consistent among the levels of the treatments GABA and refrigeration, for the respective experiments.

2.2.3.2. Water flow

Experiments 2, 3, 5, 6, 8 and 10

In experiments 2, 3, 5, 6, 8 and 10, two standard rates of water flow were investigated (Table 2.1). The first had no water flow through the down-weller for the first 18-24 hours (-flow), whilst the other had a flow of ~250 ml.min.$^{-1}$ (+flow) immediately after the addition of larvae. This treatment was investigated as rates of flow at field locations were known to be highly variable and larvae released to these locations may not successfully settle if exposed to substrata with high rates of flow.

It was hypothesised that the settlement of larvae would be significantly affected by the flow of water across the settlement substratum and that this pattern of settlement would be consistent among other treatments. In experiments 8 and 10 the proportion of settled larvae that metamorphosed and the size of settled larvae was measured (Table 2.2). These data were used to test the hypotheses that the proportion of settled larvae that metamorphosed, and the size of settled larvae was not significantly different among levels of the treatment flow and that these patterns were consistent among the levels of the treatments refrigeration and temperature shock, for the respective experiments.

2.2.4. Contributing factor - Batch

Experiments 11 & 12

The consistent use of particular treatments of factors was designed to provide a standard set of data to investigate the variation in the patterns of settlement of larvae of the same age, among different batches. It was hypothesised that there would be no significant difference in the settlement of larvae of 7 or 8 days of age, from different batches. In experiments 11 and 12 three replicates, randomly selected from a standard set of treatments (i.e. Substrata [Slides], Flow [-], GABA [1], Refrigeration [0], Temperature shock [0] and Exposure times of 60 sec. and 24 hr) were sampled from experiments 4, 5 and 10 and 2, 7, and 8, respectively (Table 2.1). Each replicate represented an independent assessment of the proportion of larvae that settled within the treatment combinations.

2.2.5. Standard experimental protocol

Larvae were sourced from captive, conditioned broodstock and reared through to settlement (i.e. 7-9 days post-fertilisation) in accordance with methods described in Heasman *et al.* (2004).

Larvae from rearing tanks were concentrated into 20 L and their total number determined from multiplying the mean number of larvae, counted from 5 replicate 2 ml samples, by 10 000. The volume required to contain approximately 20 000 larvae was decanted from the 20 L concentrate. Groups of larvae were then made up to 1 L with ambient filtered (10 µm) seawater in 1 L plastic containers. All groups of larvae were handled identically prior to, and after their exposure to different treatments. Once treated, larvae were mixed thoroughly, but gently, in the container, using a perforated plunger. A minimum of four, 1 ml sub-samples were taken from each container. The number of larvae in each sub-sample was counted in a sorting tray under a binocular microscope, and the mean used to estimate the volume of the sample required to be taken from the container to yield 200 larvae. Aliquots of the required volume were then taken from the container, using an automatic pipette with an enlarged tip, to minimise any physical damage to the larvae, and released in the designated replicate down-weller. Prior to larvae being added, the required settlement substrata was fully immersed in each replicate down-weller.

After larvae were exposed to the settlement substrata for the allocated period of time, the substrata was removed from each replicate in the down-weller using fine forceps and preserved in a jar containing 70% alcohol. The remaining content of the down-weller (i.e. larvae that had not settled on the substrata) was rinsed out and preserved separately in the same manner as that for the substrata. The number of larvae in each of the substratum and rinse samples was then counted with the aid of a binocular microscope to determine the proportion of larvae that had settled within each replicate.

The presence or absence of shell growth and the maximum length of peristomal shell was measured from a sub-sample of settled larvae, taken from within replicates of each of the five experiments described in Table 2.2. This was done to determine the effects of treatments on the competency of larvae to metamorphose and grow, respectively. Observation of shell growth and shell measurements were made with the aid of a compound microscope and image analysis software using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

2.2.6. Miniature down-welling system

An essential component of this work was the development of a method of assessing the number of abalone larvae that settle and metamorphose over a range of times in response to a number of replicated experimental treatments. To achieve this, a novel, miniature, down-welling system was designed (Plate 2.1). The design was modular, allowing the exposure of small groups of abalone larvae to replicated treatments of different experimental conditions. Each module, or bath, was capable of holding 6 rows of 4 down-welling units or replicates. Each bath had dimensions of 360 x 250 x 150 mm and had an overflow below the rim of the bath. Replicate

down-wellers consisted of an inverted 120 ml, screw top plastic jar (~40 mm dia. x 100 mm) with the base cut off, small holes drilled through each side near the base and a 35 mm hole cut out of the lid. The inside of each lid was lined with a sheet of 80 μm polyester mesh, which was secured by screwing the lid back onto the tube. Each row of 4 down-wellers was suspended in the bath on a rod threaded through the side of the bath and the top of each down-weller so that the open top of the down-weller was above the water level. Each down-weller had the water flow controlled to it through a manifold with individual taps above each down-weller. Seawater supplied to this system was from a reservoir containing filtered (5 μm), UV treated seawater at a controlled temperature of 18^{o}C.

Three modules were used to allow 72, replicated, experimental down-wellers. Where experimental designs required more than 72 replicates, treatments with short settlement times (i.e. 30 or 60 seconds) were completed first, and replaced with rows of clean down-wellers and substrata for additional replicates. Due to the constraints of this down-weller system, replicates were commonly grouped into sets of four to allow the introduction of larvae and subsequent sampling, whilst not interfering with other groups of replicates. Groups of replicates for each treatment in each experiment were randomly assigned to one of the 3 baths and there were at least two baths per treatment, unless otherwise described in specific experiments above.

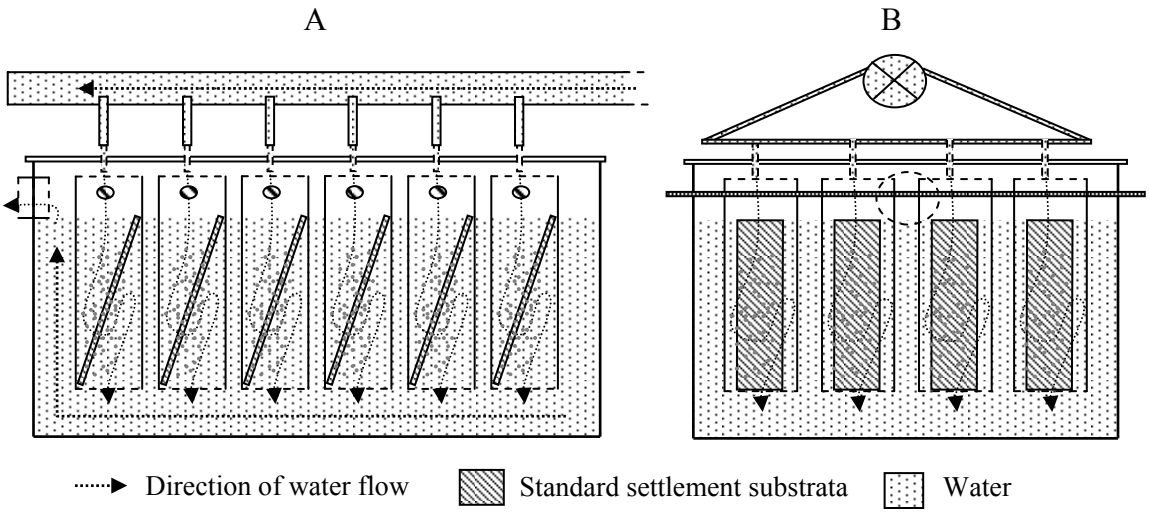


Plate 2.1 A schematic diagram (not to scale) of the side (A) and end (B) profile of one of the three modules of the miniature down-welling system containing six rows of four suspended down-welling units, each containing a standard settlement substratum. Arrows indicate the direction of water flow.

Data analysis

All of the experiments were orthogonal designs. The settlement of larvae was measured as the proportion of larvae on the settlement substrata after the designated exposure time (Table 2.1).

Metamorphosis of settled larvae equalled the proportion of a sub-sample of settled larvae (~20%; Table 2.2) that had peristomal shell growth. Size measurements (mm x 10^{-3}) were the maximum length of the peristomal shell. Cochran's *C*-test was used to test for heterogeneity of variances prior to transformation and proportional data was transformed using the function arcsine-square root (Underwood 1997). Analysis of variance (ANOVA) was used to test for differences among treatments. In each of the models all treatments were analysed as fixed factors, with the exception of Experiments 11 and 12, where Batch was a random factor.

Table 2.1 Design of experiments investigating the settlement of *H. rubra*. Treatments for each factor are shown in brackets and described in the text. Letters attached to experiment numbers indicate the use of the same batch of larvae, as applied within this chapter only. A replicate consists of an individual down-weller containing ~200 larvae (described in the text).

Expt. No.	Factors [and treatments]	Age (days)	Replicates	Exposure time						
				sec.			min.	hr.		
1a	Substrata [Slides, Rocks]	8	4		60		30	24		96
2b	Substrata [Slides, Rocks] Flow [+, -]	8	3	30	60		30	24		
3b	Substrata [Slides, Rocks] Flow [+, -] Refrigeration [0, 24]	9	3		60		30	24		
4c	GABA [0, 1, 2]	7	8		60			24	48	
5d	GABA [0, 1] Flow [+, -]	7	3	30	60		30	24		
6d	GABA [0, 1] Flow [+, -] Refrigeration [24]	8	3	30	60		30	24		
7c	Refrigeration [0, 24]	8	4		60			24	48	
8e	Refrigeration [0, 24, 48] Flow [+, -]	8	3		60		30	24	48	
9a	Temperature shock [0, 5, 10]	7	4		60		30	24		96
10e	Temperature shock [0 ,5] Flow [+, -]	7	3		60		30	24	48	
11	Batch [c, d, e]	7	3		60			24		
12	Batch [b, c, e]	8	3		60			24		

Table 2.2 Components of experiments described in Table 2.1 used to investigate the metamorphosis and difference in size of settled *H. rubra*. Treatments for each factor are shown in brackets and described in the text. Letters attached to experiment numbers indicate the use of the same batch of larvae, as described within this chapter only. A replicate consists of an individual down-weller containing ~200 larvae (as described in the text). The mean (SE) number of larvae measured, to determine the proportion metamorphosed and mean peristomal shell length per replicate, is shown.

Expt. No.	Factors [and treatments]	Age (days)	No. replicates sampled	Mean (SE) No. larvae measured per replicate	Exposure time hr.		
1a	Substrata [Slides, Rocks]	8	4	17 (1)			96
4c	GABA [0, 1, 2]	7	4	15 (0.9)	24	48	
8e	Refrigeration [0, 24, 48] Flow [+, -]	8	3	19 (0.5)	24	48	
9a	Temperature shock [0, 5, 10]	7	3	14 (0.6)			96
10e	Temperature shock [0, 5] Flow [+, -]	7	3	16 (0.8)		48	

2.3. RESULTS

2.3.1. Settlement substratum

Experiment 1

In this experiment the magnitude of the difference in the proportion of larvae that settled varied between substratum type and by time, resulting in a significant interaction term (Table 2.3). However, there were large differences in the settlement of larvae between the two settlement substrata (Figure 2.1). The proportion of larvae settling on CCA-coated rocks was generally double that to a standard settlement substratum, and a greater proportion of larvae settled after being exposed to a substratum for longer periods of time. More than 75% of larvae settled on CCA-coated rocks within 30 minutes (Figure 2.1). Settlement substrata had no significant effect on the proportion of settled larvae that metamorphosed after 96 hours (Table 2.4) and, notably, the proportion of settled larvae that metamorphosed in response to either substratum was over 80% (Figure 2.1). Substratum type also did not have a significant effect on the size of settled larvae after 96 hours (Table 2.5), with the average length of peristomal shell being 0.05 (± 0.005 SE) mm and 0.06 (± 0.006 SE) mm on the standard settlement substratum and CCA-coated rocks, respectively.

Experiment 2

There were significant differences in the proportion of larvae that settled among substratum types, but the magnitude of these differences varied among exposure times (Table 2.6). However, the proportion of larvae that settled among levels of the factor flow were consistently and significantly different (Table 2.6). Substratum types with zero flow had a greater proportion of settled larvae compared with the same substratum with flow (Figure 2.2). Notably, the proportion of larvae settling on CCA-coated rocks was more than double that to the standard settlement substratum (Figure 2.6), as observed in experiment 1.

Experiment 3

Differences in the proportion of larvae that settled among substratum types through time were inconsistent among treatments of the factors flow and refrigeration (Table 2.7). These differences were evidenced by differences in the magnitude of the proportion of larvae that settled between substratum types through time among treatments of flow, and differences in the proportion of settled larvae within substratum types through time among treatments of refrigeration (Figure 2.3). Notably, greater than 50% of larvae settled on a standard settlement substratum after 24 hours in zero flow, with greater than 80% settling after being exposed to 24 hours of refrigeration (Figure 2.3). Further, greater than 25% and 80% of larvae settled on a

standard settlement substrata and CCA-coated rocks after 24 hours, respectively, irrespective of levels of refrigeration and flow.

2.3.2. Factors relating to handling and transport

2.3.2.1. Gama aminobutyric acid (GABA)

Experiment 4

Different concentrations of GABA resulted in minor differences in the settlement of larvae. In experiment 4, the proportion of larvae that settled in response to different concentrations of GABA differed significantly through time (Table 2.8). However, irrespective of the treatment of GABA >50% of all larvae settled after at least 24 hours, and up to 86% settled after 48 hours (Figure 2.4). Less than 15% of larvae settled within 60 seconds of being exposed to the settlement substrata. However, the proportion of larvae that settled after 60 seconds was greater where GABA had been added (9% and 13% for 1 x 10^{-6}M and 2 x 10^{-6}M, respectively, compared with 6% for the control).

The proportion of settled larvae that metamorphosed through time was not consistent among treatments of GABA (Table 2.9). However, the proportion of settled larvae that had metamorphosed after 24 hours, irrespective of experimental treatment, was over 80%, with the exception of larvae that were not exposed to GABA and had 48 hours to settle (Figure 2.4).

Different concentrations of GABA had no consistent effect on the size of settled larvae through time (Table 2.10). Notably, after 48 hours on the settlement substrata, the size of larvae that were not exposed to GABA was similar to that of larvae after only 24 hours. This also coincided with only 51% of settled larvae having metamorphosed in this treatment, compared with all other treatment combinations where >80% of larvae metamorphosed. However, larvae from all treatments did show similar total lengths after 24 hours, and those exposed to GABA after 48 hours were up to twice the length of larvae exposed for only 24 hours (Figure 2.5).

Experiment 5

The affect of GABA on the proportion of larvae that settled through time differed significantly with flow (Table 2.11). Different proportions of larvae settled between treatments of the factors GABA and flow through time resulting in a significant interaction (Table 2.11 and Figure 2.6). Notably, more than twice as many larvae settled after 30 and 60 seconds with the addition of GABA and without flow (Figure 2.6).

Experiment 6

A greater proportion of larvae settled in response to exposure to GABA irrespective of patterns of settlement through time that differed significantly with treatments of flow (Table 2.12 and

Figure 2.7). More than double the proportion of larvae settled with the addition of GABA for times of 60 seconds or less (Figure 2.7). Irrespective of the treatments of GABA greater than 25% more larvae settled in no flow for settlement times of 30 minutes or more (Figure 2.7).

2.3.2.2. Refrigeration

The effect of the refrigerated storage on the proportion of larvae that settled was complicated by other factors within experiments 3, 7 and 8.

Experiment 3

In experiment 3, as described above, there were significant differences in the proportion of larvae that settled within substratum types through time that differed among treatments of refrigeration (Table 2.7). However, irrespective of treatments of refrigeration, greater than 50% of larvae settled after 24 hours on either substratum where there was no flow and, where larvae were refrigerated, this proportion was greater than 80% (Figure 2.3).

Experiment 7 and 8

In experiment 7 and 8 the magnitude of the difference in the proportion of larvae that settled through time was not consistent among treatments of refrigeration (Table 2.13 and 2.14) and, in experiment 8, differences in the proportion of larvae that settled through time, within treatments of refrigeration were also inconsistent among treatments of flow (Table 2.14). In both experiments about twice as many larvae settled after 60 seconds after being refrigerated for at least 24 hours, with this proportion almost doubling again after 48 hours of refrigeration. Consistent with results from experiment 3, about 75% of larvae settled after 24 and 48 hours in the absence of flow, irrespective of treatments of refrigeration (Figures 2.8 and 2.9).

In experiment 8, there was no significant effect of refrigeration on the proportion of settled larvae that metamorphosed, although there was a significant effect of time (Table 2.15). Between 84% and 93% of settled larvae metamorphosed after 24 hours, and between 91% and 100% metamorphosed after 48 hours (Figure 2.9). Also, in experiment 8 there were significant differences in the size of settled larvae related to refrigeration that changed through time and among treatments of flow (Table 2.16). Larvae exposed to longer periods of refrigeration tended to grow to a smaller size than larvae that were not refrigerated, or larvae that were refrigerated for a shorter period of time, although this trend tended to be more substantial for larvae exposed to the longer period of refrigeration (Figure 2.10).

2.3.2.3. Temperature shock

Experiments 9 &10

There was no significant difference in the proportion of larvae that settled in relation to treatments of temperature shock investigated in experiments 9 and 10 (Tables 2.17 and 2.20). These treatments included increases in water temperature of up to 10^{o}C over ~30 minutes, followed by immediate exposure to the settlement substrata in seawater of the original temperature (18^{o}C). In both experiments the proportion of larvae on the settlement substrata was significantly different through time and in experiment 10 this was also consistent within the factor flow (Tables 2.17 and 2.20). A greater proportion of larvae settled on a standard substrata through time and in no flow (Figures 2.11 and 2.13).

There was no significant effect of temperature shock on the proportion of settled larvae that metamorphosed in experiment 9 (Table 2.18), with greater than 90% of all larvae that settled after 96 hours having undergone metamorphosis (Figure 2.11). However, in experiment 10, the proportion of settled larvae that metamorphosed was significantly affected by temperature shock, although this effect was not consistent among the factor flow (Table 2.21). Notably, the proportion of settled larvae that metamorphosed was over 80% irrespective of the different treatment combinations, with the exception of larvae exposed to flow and no temperature shock, where the proportion of metamorphosed larvae was ~70% (Figure 2.13).

In experiment 9, temperature shock did have a significant effect on the size of settled larvae after 96 hours (Table 2.19). Notably, larvae exposed to a 5^{o}C shock were on average more than 0.02 mm larger than larvae not exposed to a temperature shock, although this difference was substantially less for larvae exposed to a 10^{o}C shock (Figure 2.12). Similarly, in experiment 10 there was a significant difference in the size of settled larvae after exposure to a temperature shock but this difference was not consistent among treatments of flow (Table 2.22). Larvae exposed to a 5^{o}C temperature shock in experiment 10 were ~0.02 mm larger than larvae not exposed to a temperature shock and held in flow, whereas they were ~0.02 mm smaller than larvae not exposed to a temperature shock and held in no flow (Figure 2.14).

Notably, in both experiment 9 and 10 the proportion of larvae that settled, metamorphosed and grew and that were exposed to a temperature shock indicate that they are capable of settlement, development and growth, at least within the bounds of the treatments investigated in these experiments.

2.3.3. Factors imposed following release

2.3.3.1. Exposure time

All experiments

There were large and consistent differences in the proportion of larvae that settled through time. In both experiments 9 and 10 a significantly greater proportion of larvae settled within treatments of the factor time with this pattern of settlement being consistent among the treatments of temperature shock (Tables 2.17 and 2.20) and flow (Table 2.20), respectively. Consistently lower proportions of larvae settled within short periods of time than after longer periods, with commonly less than 10% of larvae settling within 60 seconds (Figures 2.11 and 2.13) In all other experiments in this series, the specific effect of time on the proportion of larvae that settled was complicated by inconsistencies in settlement among other factors. However, commonly in excess of 75% of larvae settled after 24 hours or more, where settlement was not effected by flow. Also, irrespective of other factors or their combinations, the proportion of larvae that settled within 24 hours was often 4-10 times higher than the proportion that settled within 60 seconds. For example, in experiments 4 and 8, despite significant interaction terms between the factors time and GABA, and flow and refrigeration, respectively, (Tables 2.8 and 2.14) the proportion of larvae that settled after 24 hours was between 6-10 and 4-80 times greater than that after only 60 seconds, respectively (Figures 2.4 and 2.9). Moreover, in experiments 2 and 3 the proportion of larvae that settled within 60 seconds to CCA-coated rocks approached that for the standard settlement substrata over 24 hr (Figures 2.2 and 2.3).

In experiment 8, the time larvae were exposed to a standard settlement substrata did significantly affect the proportion of settled larvae that metamorphosed (Table 2.15). About 80% of settled larvae had metamorphosed after 24 hours and about 10% more had metamorphosed after 48 hours. In experiment 4, the proportion of settled larvae that metamorphosed was significantly affected by the time larvae were exposed to the settlement substrata, although this effect was not consistent among the levels of the treatment GABA (Table 2.9). However, similarly to results in experiment 8, more than 80% of settled larvae had metamorphosed after 24 hours (Figure 2.4).

In experiment 4 the size of larvae was again significantly affected by time but this effect was not consistent among treatments of GABA (Table 2.10). In experiment 8, discerning the effect of time on the size of larvae was complicated by the effects of refrigeration and flow (Table 2.16). Notably however, within experimental treatments larvae tended to show an increase in mean size between 24 and 48 hours in both experiments, irrespective of the effects of other factors (Figures 2.5 and 2.10).

2.3.3.2. Water flow

Experiments 2, 3, 5, 6, 8 and 10

There were large and consistent differences in the settlement of larvae related to the factor flow. In both experiments 2 and 10 the proportion of larvae that settled was significantly affected by the factor flow, with this being consistent among treatments of substratum and time (Table 2.6) and temperature shock and time (Table 2.20), respectively. In each of these experiments the proportion of larvae that settled without flow, within 24 hours was at least 50% greater than the proportion of larvae that settled in the presence of water flow. For the remaining experiments that included treatments for the factor flow, i.e. experiments 3, 5, 6 and 8, (described previously) consistency in the proportion of larvae that settled in response to water flow was complicated by interactions with other factors. Despite these inconsistencies the proportion of larvae that settled in response to no flow tended to be substantially greater within treatment combinations. For example, in experiment 5, despite the differences in the proportion of larvae that settled between 30 and 60 seconds among treatments of GABA, and the difference in the magnitude of the proportion that settled among GABA treatments between 30 minutes and 24 hr, the proportion of larvae that settled in the absence of flow was greater within all treatment combinations, with the exception of the 30 second treatment without GABA (Table 2.11 and Figure 2.6).

In experiment 8, treatments of the factor flow had no significant effect on the proportion of settled larvae that metamorphosed (Table 2.15) whereas in experiment 10 the effect of treatments of the factor flow on the proportion of settled larvae that metamorphosed, was not consistent among treatments of temperature shock (Table 2.21). However, in both experiments the proportion of settled larvae that metamorphosed was greater than 80% and 70% respectively.

The effect of flow on the size of settled larvae after 24 and 48 hours in experiment 8 and after 48 hours in experiment 10 was not consistent among treatments of the factors refrigeration and time, and temperature shock, respectively (Tables 2.16 and 2.22). However, the size of larvae, particularly within the first 24 hours of settlement tended to be greater in the absence of water flow, with the mean size of larvae more than halved in the presence of water flow. The effect of water flow on the size of settled larvae after 48 hours tended to be substantially less than over 24 hours (Figures 2.10 and 2.14).

2.3.4. Contributing factor - Batch

Experiments 11 & 12

The difference in the proportion of 7 and 8 day old larvae that settled within 60 sec. and 24 hours differed among batches in both experiments 11 and 12 respectively, resulting in a significant interaction term between the factors time and batch (Tables 2.23 and 2.24). In some instances the proportion of larvae of either age that settled after 60 sec. or 24 hours was more than double that of larvae from other batches. However, the major difference in the proportion of larvae that settled was between 60 seconds and 24 hours. Less than 20% of 7 day old larvae, and less than 10% of 8 day old larvae settled after 60 seconds and greater than 50% of 7 day old larvae and 35% of 8 day old larvae settled after 24 hours in experiments 11 and 12 respectively (Figures 2.15 and 2.16).

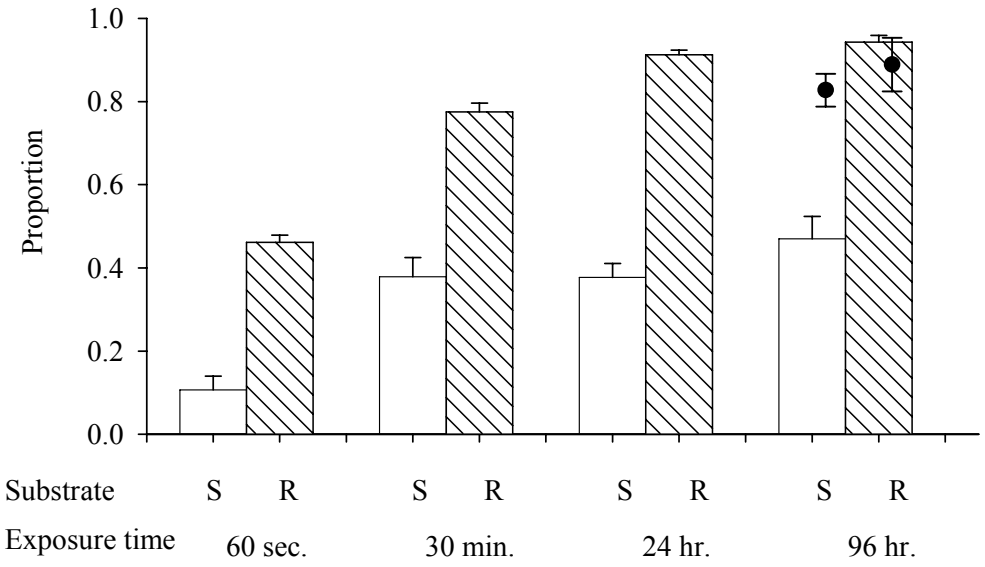


Figure 2.1 Experiment 1. Mean proportion of larvae (+ SE) on a standard settlement substratum (S) and CCA rocks (R) (histogram) for 4 exposure times, and mean proportion (± SE) of settled larvae that metamorphosed on the respective substrata (dot plot) after 96 hours; $n = 4$.

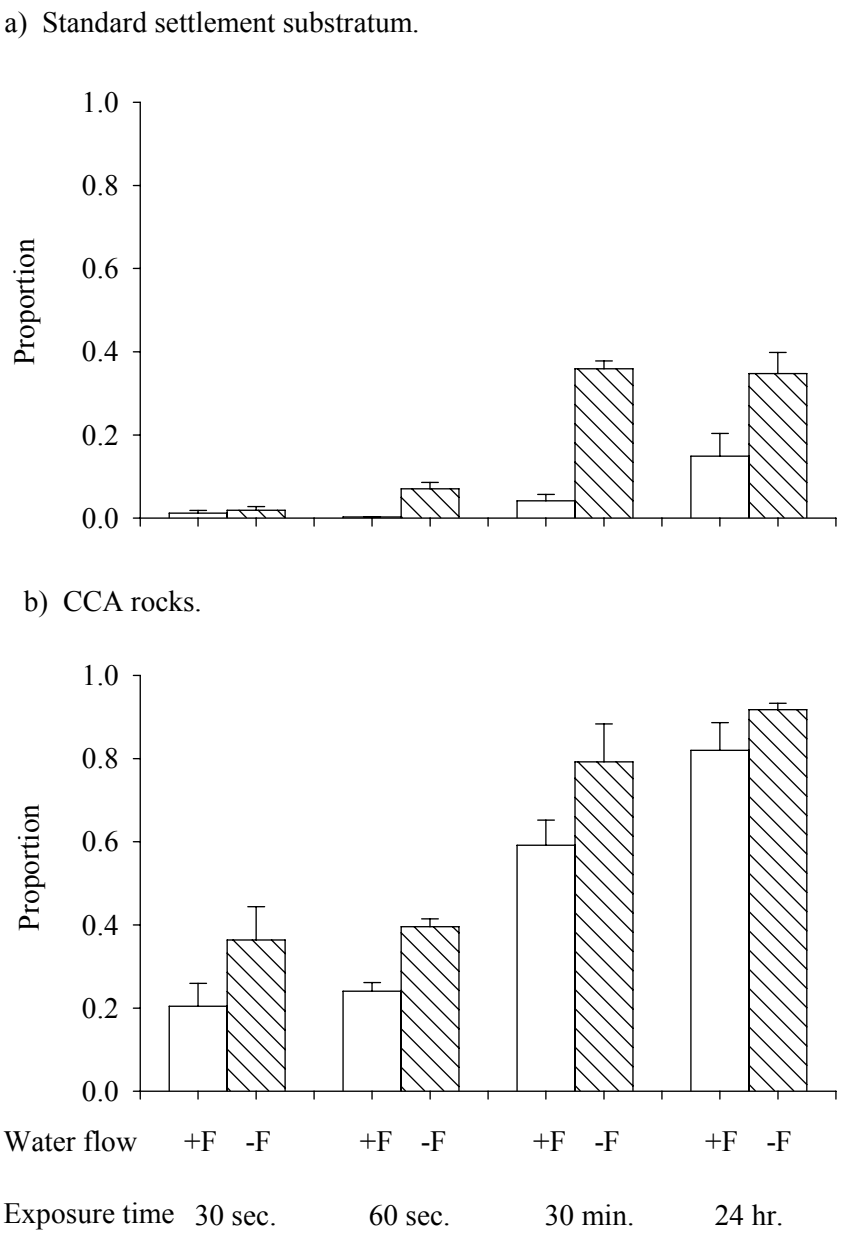


Figure 2.2 Experiment 2. Mean proportion of larvae (+ SE) on a standard settlement substratum and CCA rocks, with and without water flow (F) for 4 exposure times; $n = 3$.

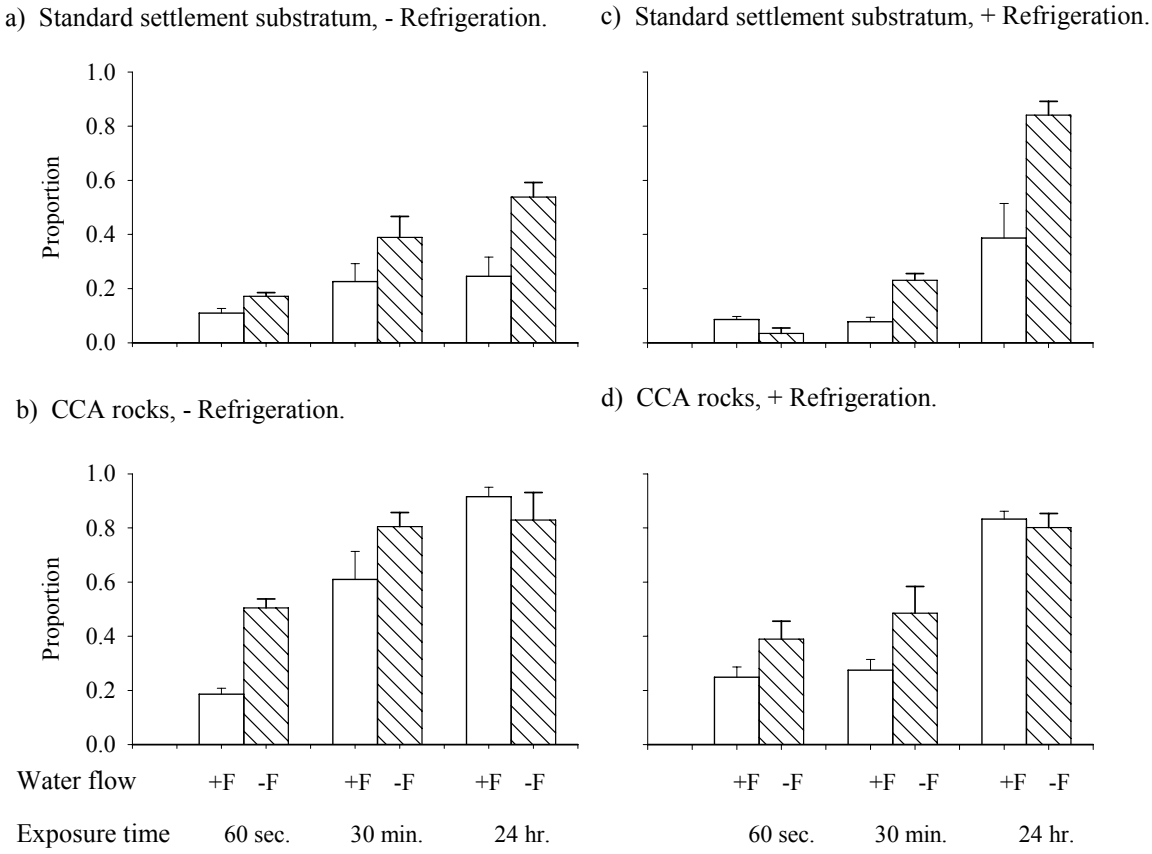


Figure 2.3 Experiment 3. Mean proportion of larvae (+ SE) on a standard settlement substratum and CCA rocks, with and without 24 hours of refrigeration and water flow (F), for 3 exposure times; $n = 3$.

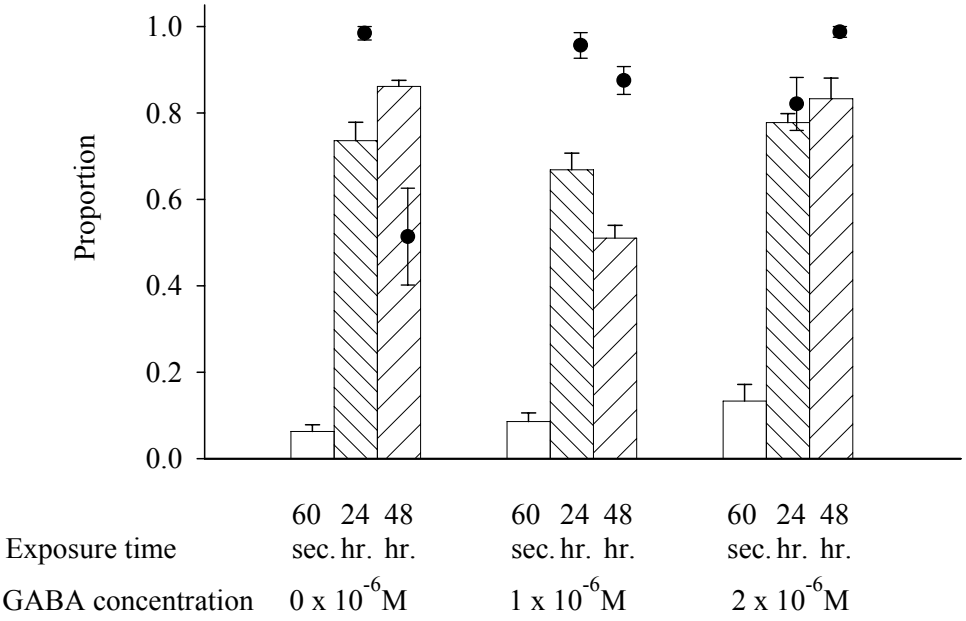


Figure 2.4 Experiment 4. Mean proportion of larvae (+ SE) on a standard settlement substratum after exposure to a control and 2 concentrations of GABA for 3 exposure times (histogram), and mean proportion of settled larvae (± SE) that metamorphosed (dot plot) after 24 and 48 hours; n = 8 and 4, respectively.

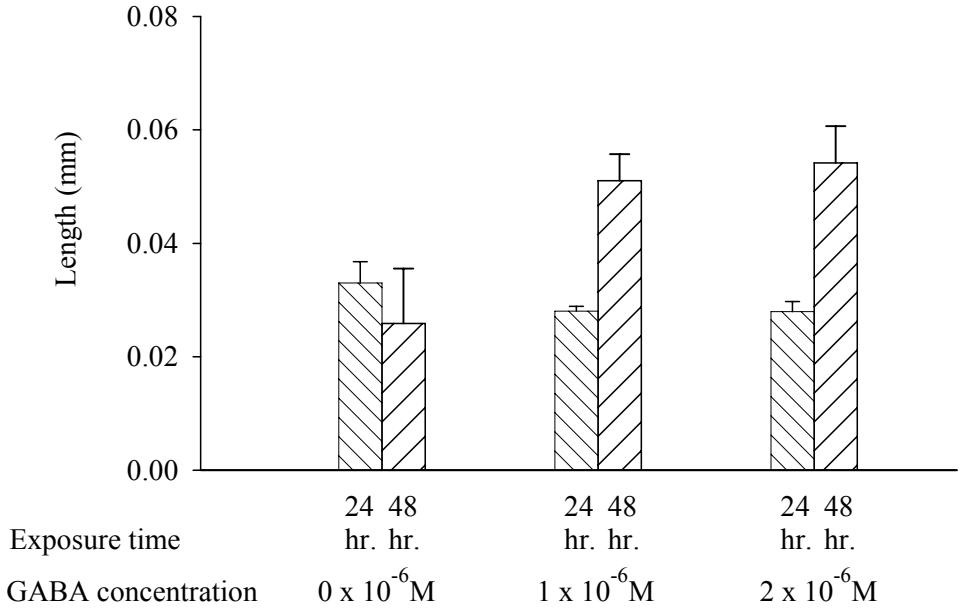


Figure 2.5 Experiment 4. Mean peristomal shell length (mm) (+ SE) of settled larvae after exposure to a control and 2 concentrations of GABA (M) for 2 exposure times; n = 4.

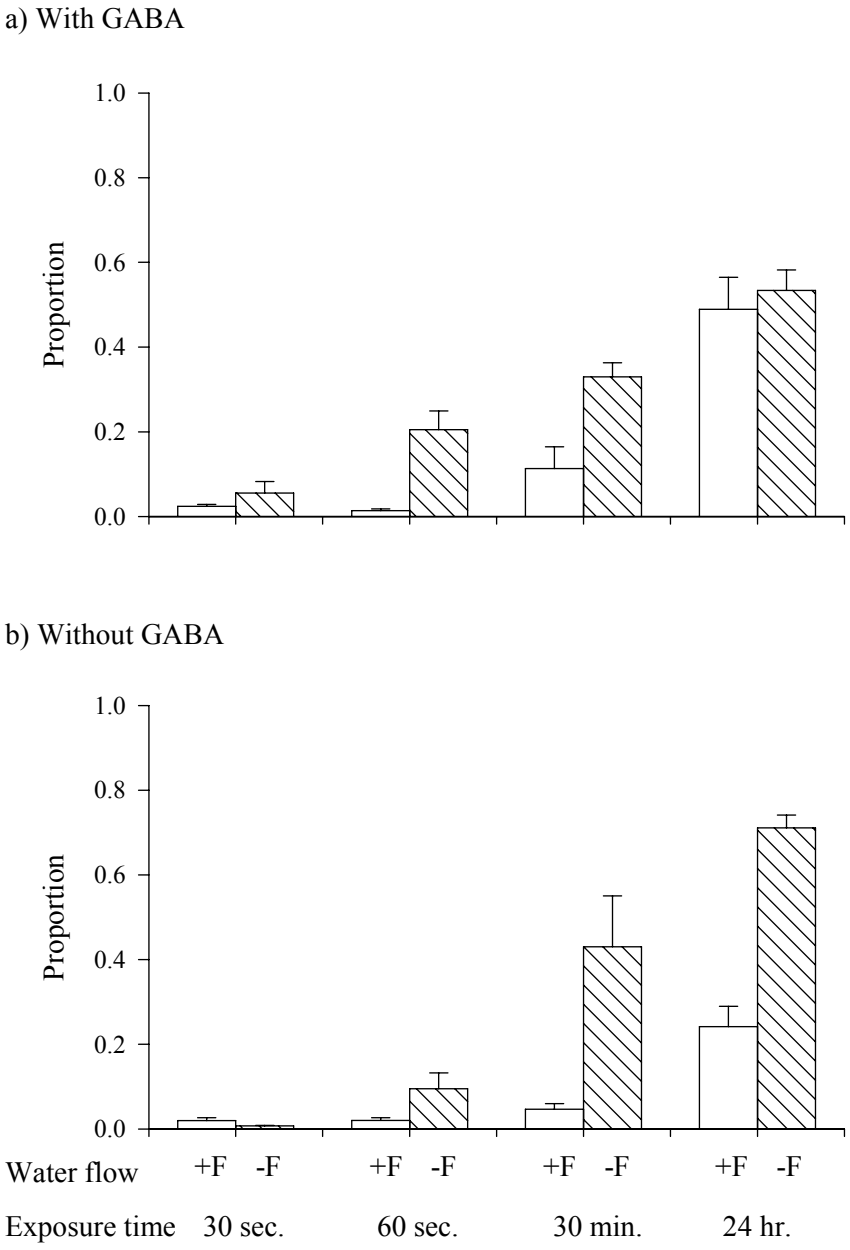


Figure 2.6 Experiment 5. Mean proportion of larvae (+ SE) on a standard settlement substrata with and without exposure to GABA (1 x 10^{-6} M) and water flow (F) for 4 exposure times; $n = 3$.

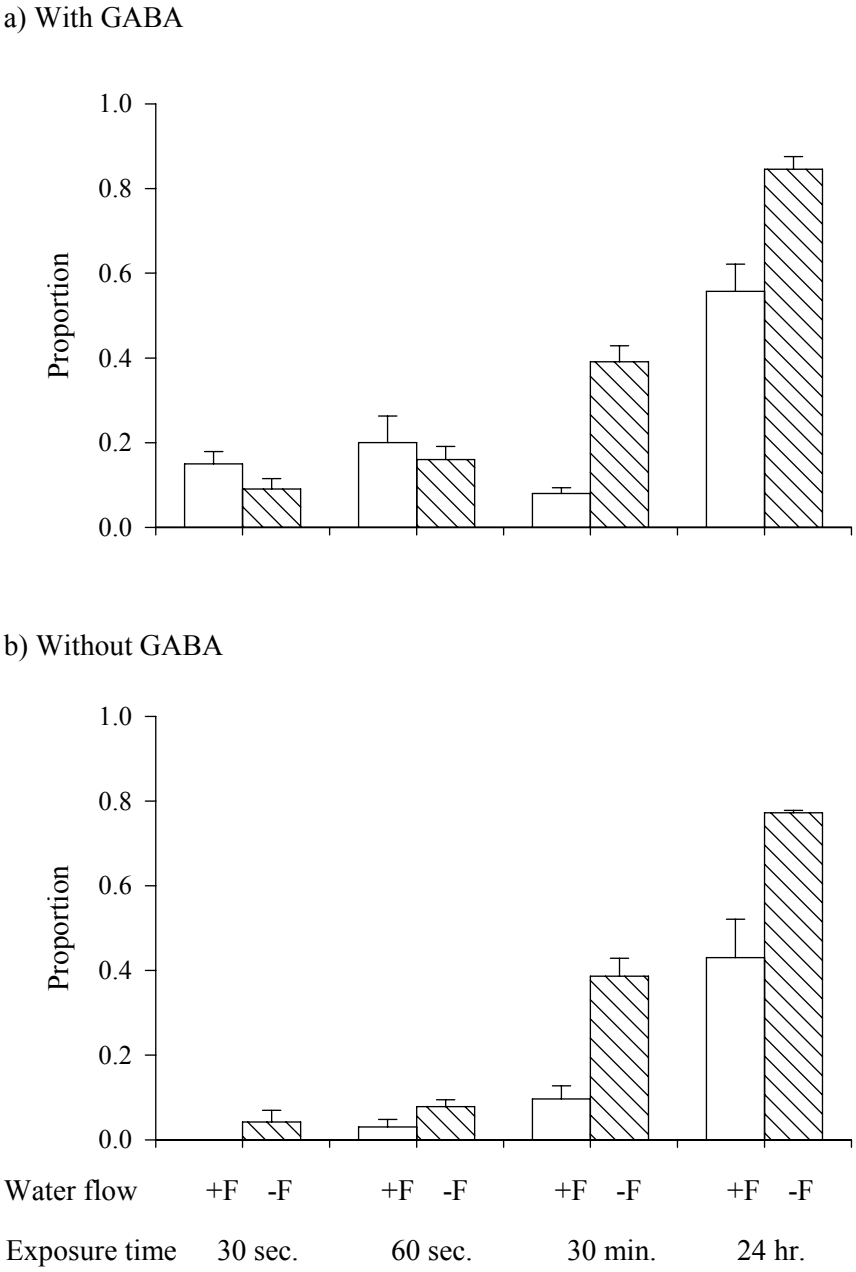


Figure 2.7 Experiment 6. Mean proportion of larvae (+ SE) on a standard settlement substrata after 24 hours of refrigeration, with and without GABA (1 x 10^{-6} M) and water flow (F) for 4 exposure times; $n = 3$.

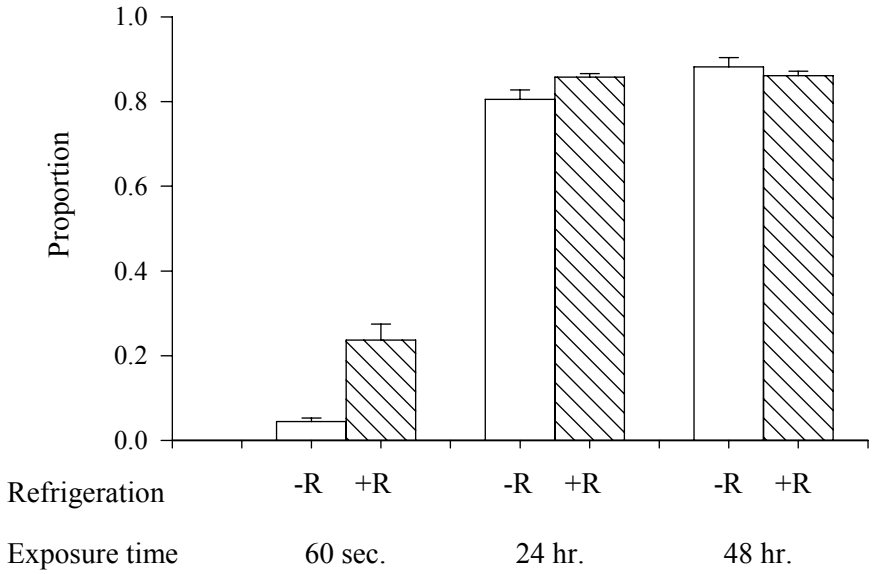


Figure 2.8 Experiment 7. Mean proportion of larvae (+ SE) on a standard settlement substrata with and without 24 hours of refrigeration (R), without water flow for 3 exposure times; $n = 3$.

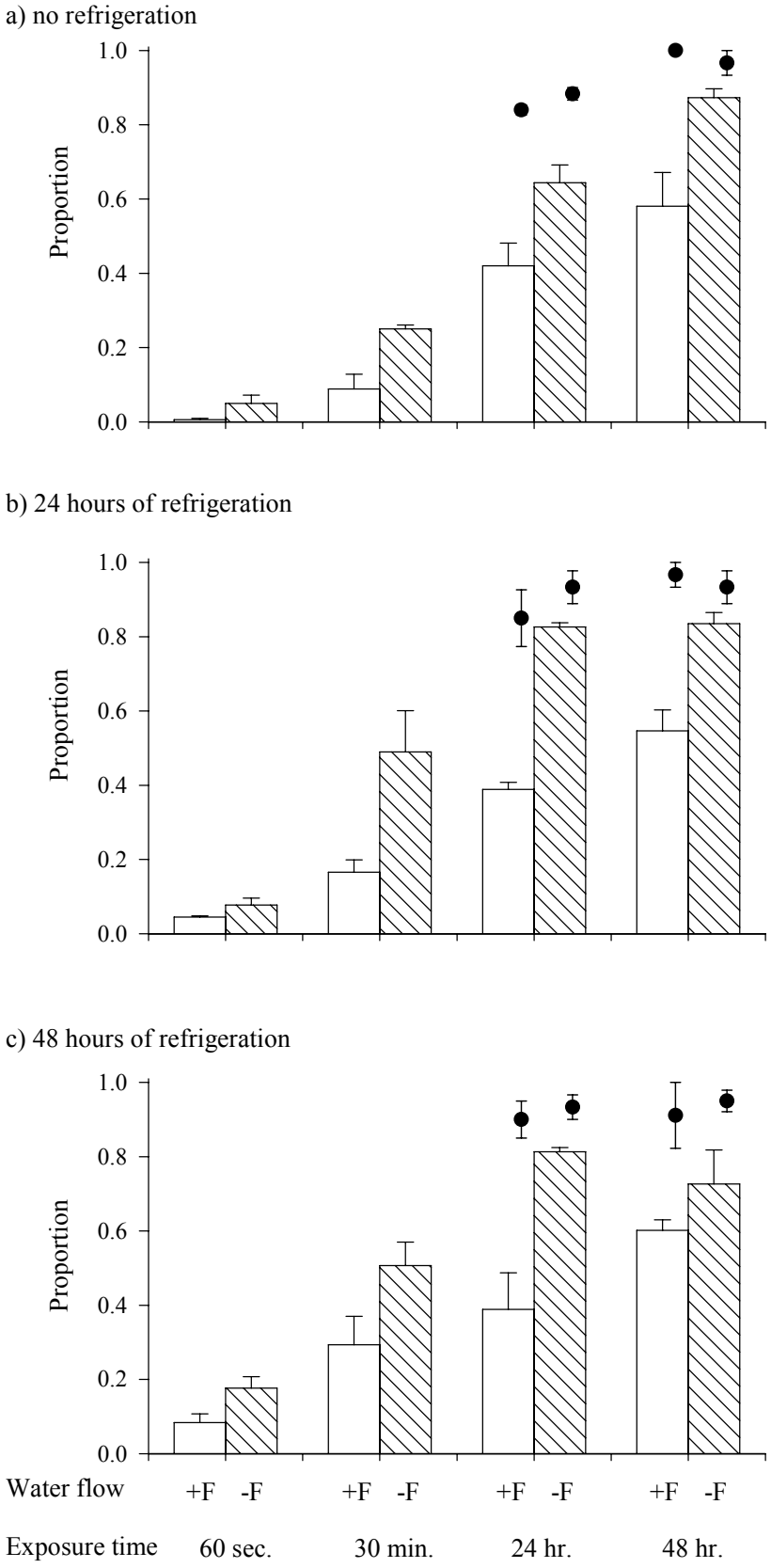


Figure 2.9 Experiment 8. Mean proportion of larvae (+ SE) on a standard settlement substratum (histogram) after 0 (control), 24 and 48 hours of refrigeration, with and without water flow (F) for 4 exposure times, and the mean proportion (± SE) of settled larvae that metamorphosed (dot plot) after 24 and 48 hours; $n = 3$.

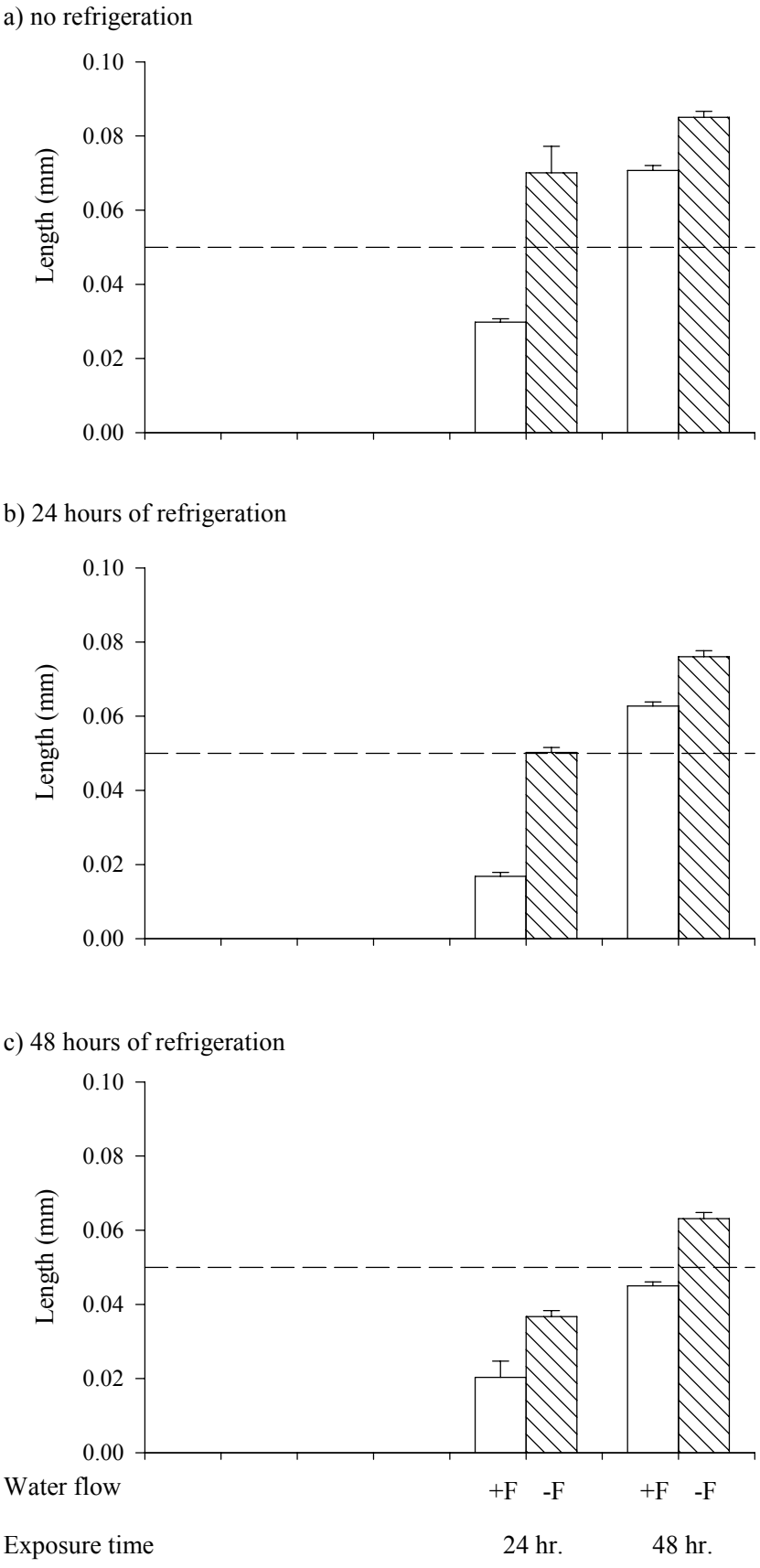


Figure 2.10 Experiment 8. Mean peristomal shell length (mm) (+ SE) of settled larvae after 0, 24 and 48 hours of refrigeration, with and without water flow (F) for 2 exposure times; $n = 3$. Dashed line is for reference and set at 0.05 mm.

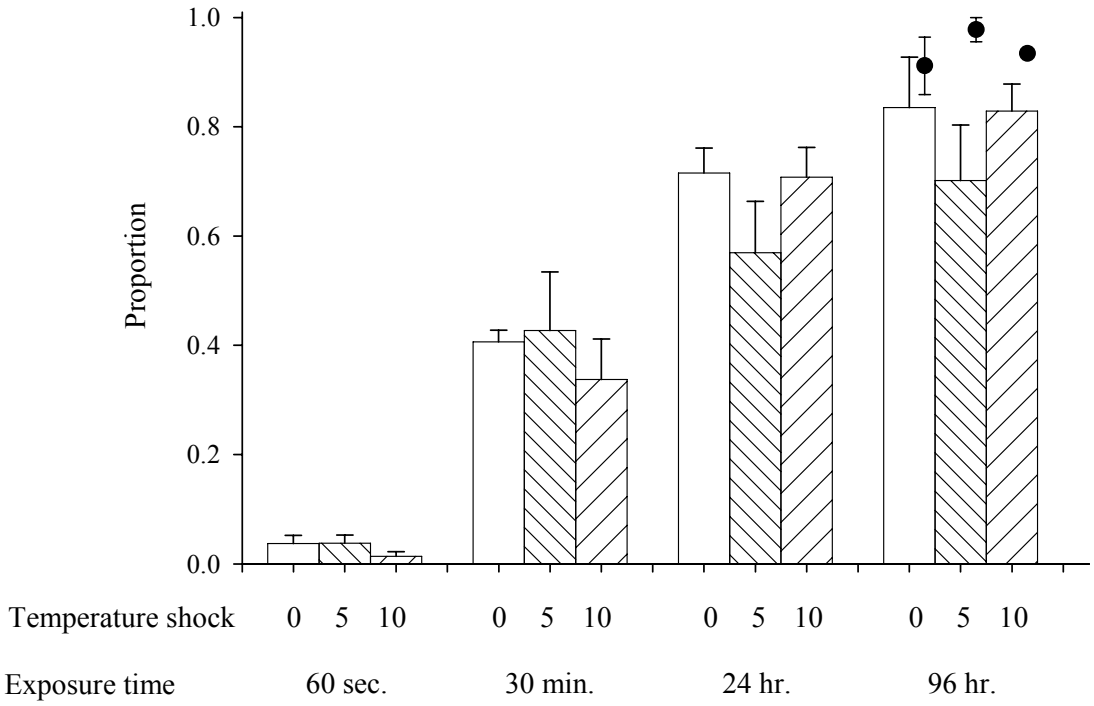


Figure 2.11 Experiment 9. Mean proportion of larvae (+ SE) on a standard settlement substratum (histogram) after a control (no temperature shock (0)) and a temperature shock of +5°C or +10°C for 4 exposure times without flow ($n = 4$), and mean proportion (± SE) of settled larvae that metamorphosed (dot plot) after 96 hours; $n = 3$.

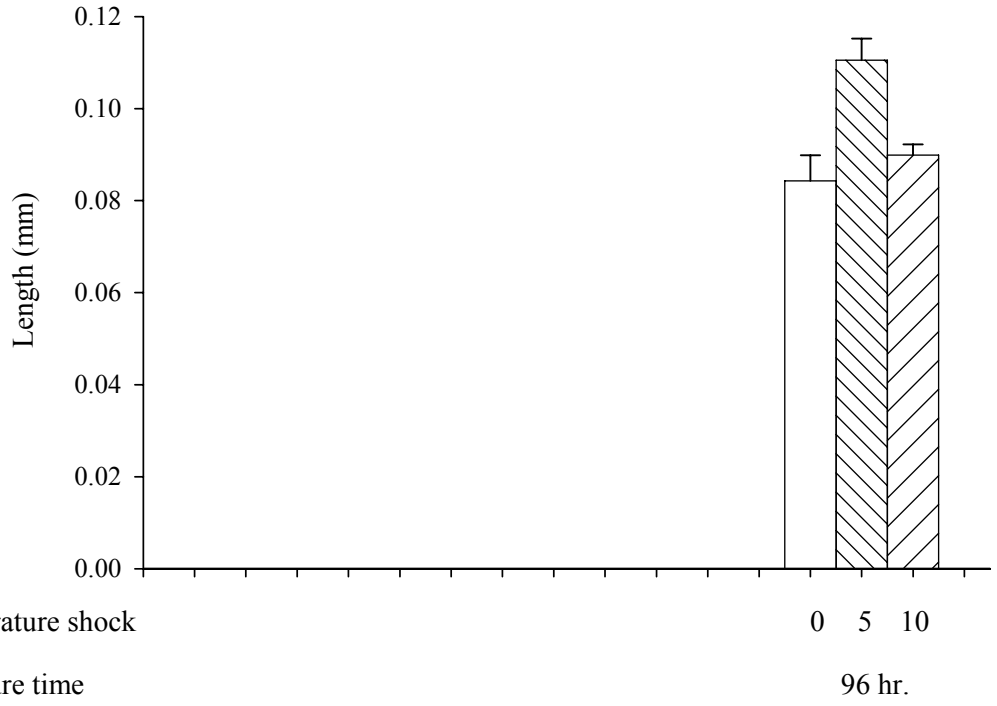


Figure 2.12 Experiment 9. Mean peristomal shell length (mm) (+ SE) of settled larvae after 96 hours on a standard settlement substratum after a control (no temperature shock (0)) and a temperature shock of +5°C and +10°C; $n = 3$.

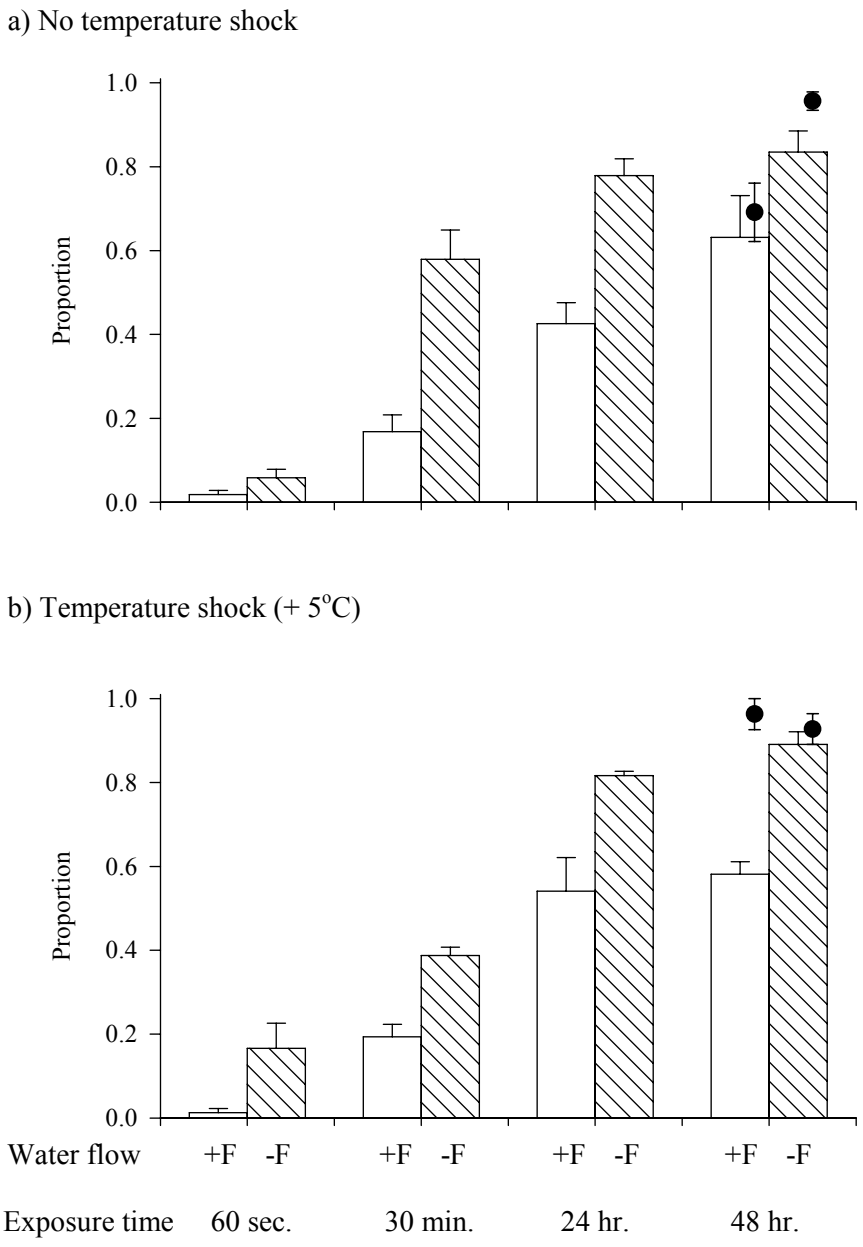


Figure 2.13 Experiment 10. Mean proportion of larvae (+ SE) on a standard settlement substratum (histogram) after a control (no temperature shock) and +5°C temperature shock, with and without water flow (F) for 4 exposure times, and mean proportion (± SE) of settled larvae that metamorphosed (dot plot) after 48 hours; $n = 3$.

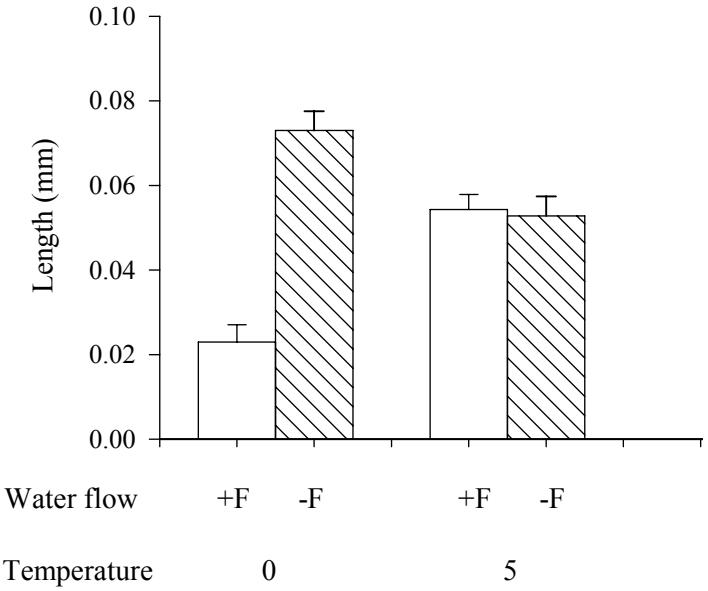


Figure 2.14 Experiment 10. Mean peristomal shell length (mm) (+ SE) of settled larvae on a standard settlement substratum after 48 hours and after a control (no temperature shock (0)) and temperature shock of +5°C, with and without water flow (F); $n = 3$.

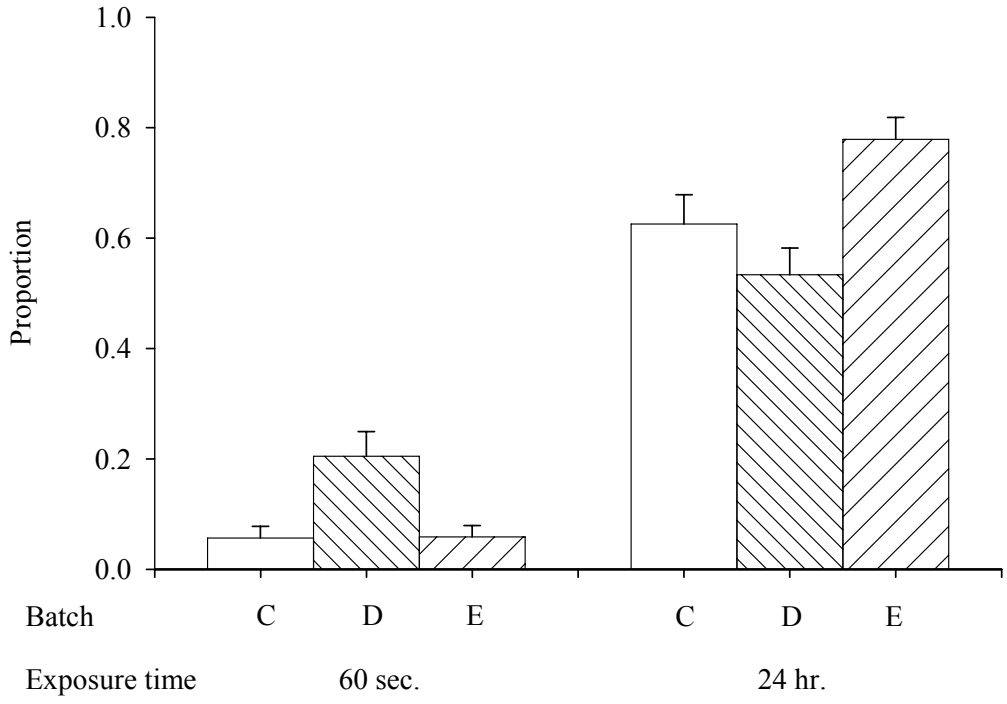


Figure 2.15 Experiment 11. Mean proportion of 7 day old larvae (+ SE) on a standard settlement substratum from 3 separate batches for 2 exposure times; $n = 3$.

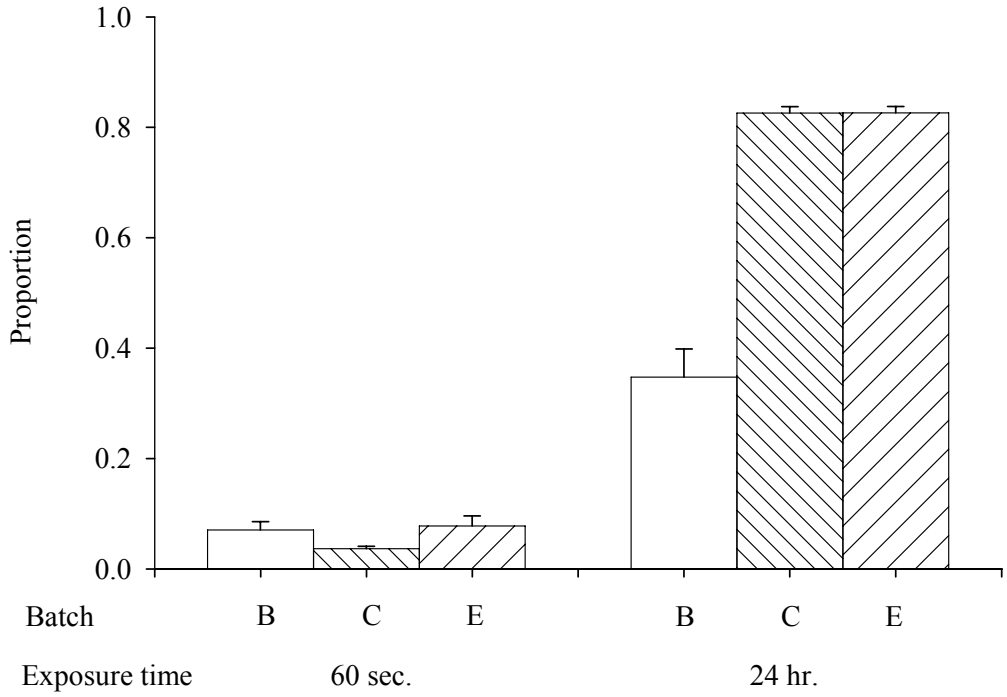


Figure 2.16 Experiment 12. Mean proportion of 8 day old larvae (+ SE) on a standard settlement substratum from 3 separate batches for 2 exposure times; $n = 3$.

Table 2.3 Experiment 1. Summary of analysis of variance in the proportion of larvae settled on two substrata (slides and CCA rocks), at different times (60 sec., 30 min. and 24 and 96 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.2630, ns; variances 8, df 3).

	Source	df	MS	*F*	F vs
1	Substrate, S	1	2.07	364.78 **	4
2	Time, T	3	0.40	71.31 **	4
3	S x T	3	0.02	3.75 *	4
4	Error	24	0.01		
5	Total	31			

Table 2.4 Experiment 1. Summary of analysis of variance in the proportion of settled larvae that metamorphosed on two substrata (slides and CCA rocks) after 96 hours. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.8867, ns; variances 2, df 3).

	Source	df	MS	*F*	F vs
1	Substrate	1	0.06	1.37 ns	2
2	Error	6	0.05		
3	Total	0			

Table 2.5 Experiment 1. Summary of analysis of variance in the size of settled larvae on two substrata (slides and CCA rocks) after 96 hours. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.6750, ns; variances 2, df 3).

	Source	df	MS	*F*	F vs
1	Substrate	1	0.0001	0.72 ns	2
2	Error	6	0.0001		
3	Total	0			

Table 2.6 Experiment 2. Summary of analysis of variance in the proportion of larvae settled on two substrata (slides and CCA rocks) with and without water flow, at different times (30 and 60 sec., 30 min. and 24 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.3003, ns; variances 16, df 2).

	Source	df	MS	*F*		F vs
1	Flow, F	1	0.53	49.96	**	8
2	Substrate, S	1	3.53	332.88	**	8
3	F x S	1	0.01	0.82	ns	8
4	Time, T	3	0.79	74.91	**	8
5	F x T	3	0.03	2.72	ns	8
6	S x T	3	0.05	4.64	**	8
7	F x S x T	3	0.01	1.41	ns	8
8	Error	32	0.01			
9	Total	47				

Table 2.7 Experiment 3. Summary of analysis of variance in the proportion of larvae settled on two substrata (slides and CCA rocks) with and without water flow and 24 hours refrigeration, at different times (30 and 60 sec., 30 min. and 24 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.1446, ns; variances 24, df 2).

	Source	df	MS	*F*		F vs
1	Flow, F	1	0.48	31.68	**	16
2	Substrate, S	1	2.20	144.97	**	16
3	F x S	1	0.02	1.26	ns	16
4	Refrigeration, R	1	0.16	10.53	**	16
5	F x R	1	0.00	0.14	ns	16
6	S x R	1	0.07	4.74	*	16
7	F x S x R	1	0.00	0.08	ns	16
8	Time, T	2	1.73	114.20	**	16
9	F x T	2	0.02	0.99	ns	16
10	S x T	2	0.01	0.74	ns	16
11	F x S x T	2	0.22	14.81	**	16
12	R x T	2	0.18	11.94	**	16
13	F x R x T	2	0.05	3.10	ns	16
14	S x R x T	2	0.09	5.95	**	16
15	F x S x R	2	0.00	0.15	ns	16
16	Error	48	0.02			
17	Total	71				

Table 2.8 Experiment 4. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substratum after exposure to different concentrations of GABA (0, $1x10^{-6}$M, $2x10^{-6}$M), at different times (60 sec., 24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.2385, ns; variances 9, df 7).

	Source	df	MS	*F*		F vs
1	GABA, G	2	0.23	17.90	**	4
2	Time, T	2	4.48	344.35	**	4
3	G x T	4	0.11	8.46	**	4
4	Error	63	0.01			
5	Total	71				

Table 2.9 Experiment 4. Summary of analysis of variance in the proportion of settled larvae that metamorphosed on a standard settlement substratum after exposure to different concentrations of GABA (0, $1x10^{-6}$M, $2x10^{-6}$M), at different times (24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.6288*;

	Source	df	MS	*F*		F vs
1	GABA, G	2	0.09	2.67	ns	4
2	Time, T	1	0.22	6.86	*	4
3	G x T	2	0.53	16.14	**	4
4	Error	18	0.03			
5	Total	23				

variances 6, df 3).

Table 2.10 Experiment 4. Summary of analysis of variance in the size of settled larvae on a standard settlement substratum after exposure to different concentrations of GABA (0, $1x10^{-6}$M, $2x10^{-6}$M), at different times (24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.6078*; variances 6, df 3).

	Source	df	MS	*F*		F vs
1	GABA, G	2	0.0003	2.71	ns	4
2	Time, T	1	0.0012	10.02	**	4
3	G x T	2	0.0007	5.75	*	4
4	Error	18	0.0001			
5	Total	23				

Table 2.11 Experiment 5. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substratum with and without exposure to GABA ($1x10^{-6}$M) and with and without water flow at different times (30 and 60 sec., 24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.2901$, ns; variances 16, df 2).

	Source	df	MS	*F*	F vs
1	Flow, F	1	0.648	65.63 **	8
2	GABA, G	1	0.027	2.69 ns	8
3	F x G	1	0.022	2.20 ns	8
4	Time, T	3	0.921	93.19 **	8
5	F x T	3	0.080	8.07 **	8
6	G x T	3	0.004	0.38 ns	8
7	F x G x T	3	0.067	6.74 **	8
8	Error	32	0.010		
9	Total	47			

Table 2.12 Experiment 6. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substratum with and without exposure to GABA ($1x10^{-6}$M) and with and without water flow at different times (30 and 60 sec., 24 and 48 hr.) after 24 hours refrigeration. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.2104$, ns; variances 16, df 2).

	Source	df	MS	*F*	F vs
1	Flow, F	1	0.286	36.55 **	8
2	GABA, G	1	0.145	18.60 **	8
3	F x G	1	0.001	0.16 ns	8
4	Time, T	3	1.121	143.48 **	8
5	F x T	3	0.177	22.60 **	8
6	G x T	3	0.022	2.76 ns	8
7	F x G x T	3	0.004	0.46 ns	8
8	Error	32	0.008		
9	Total	47			

Table 2.13 Experiment 7. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substratum after 0 and 24 hours refrigeration, at different times (60 sec., 24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.4558, ns; variances 6, df 3).

	Source	df	MS	*F*		F vs
1	Refrigeration, R	1	0.072	24.12	**	4
2	Time, T	2	1.803	600.92	**	4
3	R x T	2	0.057	18.96	**	4
4	Error	18	0.003			
5	Total	23				

Table 2.14 Experiment 8. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substratum after 0, 24 and 48 hours of refrigeration, with and without water flow, at different times (30 and 60 sec., 24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.1531, ns; variances 24, df 2).

	Source	df	MS	*F*		F vs
1	Flow, F	1	1.226	115.24	**	8
2	Refrigeration, R	2	0.098	9.22	**	8
3	F x R	2	0.008	0.79	ns	8
4	Time, T	3	2.044	192.08	**	8
5	F x T	3	0.054	5.09	*	8
6	R x T	6	0.040	3.77	*	8
7	F x R x T	6	0.016	1.51	ns	8
8	Error	48	0.011			
9	Total	71				

Table 2.15 Experiment 8. Summary of analysis of variance in the proportion of settled larvae that metamorphosed on a standard settlement substratum after 0, 24 and 48 hours of refrigeration, with and without water flow, at different times (24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.2199, ns; variances 12, df 2).

	Source	df	MS	*F*		F vs
1	Flow, F	1	0.0002	0.00	ns	8
2	Refrigeration, R	2	0.0001	0.00	ns	8
3	F x R	2	0.0017	0.05	ns	8
4	Time, T	1	0.2445	6.58	*	8
5	F x T	1	0.0496	1.33	ns	8
6	R x T	2	0.0601	1.62	ns	8
7	F x R x T	2	0.0057	0.15	ns	8
8	Error	24	0.0372			
9	Total	35				

Table 2.16 Experiment 8. Summary of analysis of variance in the size of settled larvae on a standard settlement substratum after 0, 24 and 48 hours of refrigeration, with and without water flow, at different times (24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.2406, ns; variances 6, df 3).

	Source	df	MS	*F*		F vs
1	Flow, F	1	0.0272	107.04	**	8
2	Refrigeration, R	2	0.0074	28.94	**	8
3	F x R	2	0.0001	0.55	ns	8
4	Time, T	1	0.0525	206.44	**	8
5	F x T	1	0.0065	25.56	**	8
6	R x T	2	0.0005	2.06	ns	8
7	F x R x T	2	0.0012	4.63	*	8
8	Error	24	0.0003			
9	Total	35				

Table 2.17 Experiment 9. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substratum after a control (no temperature shock (0)) and a temperature shock of 5 or 10^0C, at different times (60 sec., 30 mins and 24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.2532, ns; variances 12, df 3).

	Source	df	MS	*F*	F vs
1	Temperature, Tmp	2	0.031	1.11 ns	4
2	Time, T	3	2.215	79.08 **	4
3	Tmp x T	6	0.021	0.74 ns	4
4	Error	36	0.028		
5	Total	47			

Table 2.18 Experiment 9. Summary of analysis of variance in the proportion of settled larvae that metamorphosed on a standard settlement substratum after a control (no temperature shock (0)) and a temperature shock of 5 or 10^0C after 96 hours. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.6870, ns; variances 3, df 2).

	Source	df	MS	*F*	F vs
1	Temperature, Tmp	2	0.027	1.12 ns	2
2	Error	6	0.024		
3	Total	8			

Table 2.19 Experiment 9. Summary of analysis of variance in the size of settled larvae on a standard settlement substratum after a control (no temperature shock (0)) and a temperature shock of 5 or 10^0C after 96 hours. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.5799, ns; variances 3, df 2).

	Source	df	MS	*F*	F vs
1	Temperature, Tmp	2	0.0006	9.75 *	2
2	Error	6	0.0001		
3	Total	8			

Table 2.20 Experiment 10. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substratum after a control (no temperature shock (0)) and a temperature shock of 5 ^{0}C with and without water flow, at different times (60 sec., 30 mins and 24 and 48 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.2182, ns; variances 16, df 2).

	Source	df	MS	*F*		F vs
1	Flow, F	1	1.0739	102.17	**	8
2	Temperature, Tmp	1	0.0057	0.54	ns	8
3	F x Tmp	1	0.0001	0.01	ns	8
4	Time, T	3	1.7039	162.11	**	8
5	F X T	3	0.0088	0.84	ns	8
6	Tmp x T	3	0.0167	1.59	ns	8
7	F x Tmp x T	3	0.0284	2.70	ns	8
8	Error	32	0.0105			
9	Total	47				

Table 2.21 Experiment 10. Summary of analysis of variance in the proportion of settled larvae that metamorphosed on a standard settlement substratum after a control (no temperature shock (0)) and a temperature shock of 5^{o}C with and without water flow after 48 hours. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.3337, ns; variances 4, df 2).

	Source	df	MS	*F*		F vs
1	Flow, F	1	0.068	2.37	ns	4
2	Temperature, Tmp	1	0.132	4.57	ns	4
3	F x Tmp	1	0.205	7.12	*	4
4	Error	8	0.029			
5	Total	11				

Table 2.22 Experiment 10. Summary of analysis of variance in the size of settled larvae on a standard settlement substratum after a control (no temperature shock (0)) and a temperature shock of 5^0C, with and without water flow after 48 hours. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.4670, ns; variances 4, df 2).

	Source	df	MS	*F*	F vs
1	Flow, F	1	0.0018	33.10 **	4
2	Temperature, Tmp	1	0.0001	1.75 ns	4
3	F x Tmp	1	0.0020	37.43 **	4
4	Error	8	0.0001		
5	Total	11			

Table 2.23 Experiment 11. Summary of analysis of variance in the proportion of 7 day old larvae from 3 separate batches settled on a standard settlement substratum at different times (60 sec. and 24 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.2124, ns; variances 6, df 2).

	Source	df	MS	*F*	F vs
1	Batch, B	2	0.013	1.77 ns	4
2	Time, T	1	1.772	18.72 *	3
3	B x T	2	0.095	13.09 **	4
4	Error	12	0.007		
5	Total	17			

Table 2.24 Experiment 12. Summary of analysis of variance in the proportion of 8 day old larvae from 3 separate batches settled on a standard settlement substratum at different times (60 sec. and 24 hr.). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.5229, ns; variances 6, df 2).

	Source	df	MS	*F*	F vs
1	Batch, B	2	0.119	41.15 **	4
2	Time, T	1	2.362	15.69 ns	3
3	B x T	2	0.151	52.09 **	4
4	Error	12	0.003		
5	Total	17			

2.4. DISCUSSION

The use of hatchery-reared larvae to enhance local populations of abalone requires them to be subjected to a range of handling and transport processes. However, a number of factors prior to release may significantly affect their competency to successfully settle, survive and grow. The series of experiments presented in this chapter represent the first investigation, to the authors knowledge, into the settlement response of *Haliotis* spp to conditions they experience during release from a hatchery to a field location (e.g. refrigeration and temperature shock) and the first that investigates the combined effects of these treatments with conditions similar to those they are exposed to at a field release location (e.g. exposure time and water flow). The extent to which handling, transport and field conditions influenced metamorphosis and the size of settled larvae within 96 hours post-settlement was also measured.

Results from these experiments indicated that larvae settled on both a standard settlement substratum and CCA coated rocks and that a greater proportion settled over longer periods of time. However, larvae settled on CCA coated rocks in the laboratory at a level about twice that on a standard settlement substratum, irrespective of exposure time. GABA had a significant affect on the proportion of larvae that settled through time and among treatments of water flow. Generally, a greater proportion of larvae settled within 60 seconds after exposure to GABA. Larvae could be refrigerated during simulated transport for up to 48 hours, but refrigeration did appear to have a negative effect on growth. It is not known if the influence on growth persists beyond 2 days. Larvae appeared to be resistant to thermal shock up to 10^{o}C which would potentially be experienced during handling and transport in small volumes of seawater, to field release locations. The effect of thermal shock on size was inconsistent and appeared to be small. The time larvae were exposed to a substratum had a significant effect on the proportion that settled and metamorphosed, and their size. Commonly less than 15% of larvae settled after 60 seconds. Water flow had a consistent affect on the proportion of larvae that settled, with greater proportions of larvae settling in reduced flow. There was variation among batches in the proportion of larvae that settled, but these differences were relatively insubstantial.

The settlement response, including metamorphosis and growth, of *Haliotis* spp has been described for a broad range of chemical and biological factors (see reviews by McShane 1992, Morse 1992, Kawamura 1996, Roberts 2001). Despite the fact that there are factors (e.g. GABA) that illicit similar settlement responses among many species of abalone, there is significant variation in these responses among batches of the same species, and in the presence of other factors. In addition, the settlement response of abalone can be significantly affected by combinations of these factors (Roberts 2001), consistent with the findings for *H. rubra* larvae from the experiments done in this chapter.

2.4.1. Settlement substrata

The settlement response of *Haliotis* spp, to specific substrata is highly variable and larvae have been shown to display significant preference to specific substratum (Daume *et al.* 1999, Roberts *et al.* 2004). In particular, there is a significant amount of published data describing the strong settlement response of *Haliotis* spp to CCA (Morse & Morse 1984, Moss & Tong 1992a, Shepherd & Daume 1996, Daume *et al.* 1999, Daume *et al.* 2000, Roberts *et al.* 2004, Huggett *et al.* 2005). Of these studies comparatively little work has focused on the settlement response of *H. rubra*. However, Daume *et al.* (1999, 2000) described a relatively limited response of *H. rubra* to CCA and a high response to the encrusting green alga, *Ulvella lens*. In contrast, Huggett *et al.* (2005) described a relatively high settlement response of *H. rubra* to CCA, in addition to two species of green macroalgae.

Results from experiments 1-3 demonstrated a high settlement response of *H. rubra* to CCA, similar to that described by Hugget *et al.* (2005) and, despite the significant differences in the proportion of larvae that settled among the substrata through time in all three experiments, the general response of larvae to both CCA and a standard settlement substratum was very similar when exposed to a range of other treatments. The proportion of *H. rubra* that settled to a standard settlement substratum, without flow after 24 hours was over 30% and often over 75%. These results compare favourably to those reported elsewhere for *H. rubra* on substrata considered to be reliably good settlement inducers (Daume *et al.* 2000, Huggett *et al.* 2005). Moreover, assessment of metamorphosis of the settled *H. rubra*, described in this chapter, generally indicated rates of metamorphosis in excess of 80%.

There was no significant effect of settlement substrata on the proportion of settled larvae that metamorphosed or the size of settled larvae after 96 hours. In fact, due to the relatively short periods of time (<100 hr.) over which the experiments in this series were conducted it is unlikely that the algal composition of the standard settlement substratum would have a significant influence on the growth of settled larvae, as nutrient stores from residual yolk and dissolved organic material absorption is more likely to sustain a regular rate of growth under standard conditions (Kawamura *et al.* 1998). It is, therefore, more likely that differences in the size of settled larvae among factors is a result of experimental treatments rather than composition of the settlement substrata.

2.4.2. Factors relating to handling and transport

2.4.2.1. GABA

The affect of GABA on the settlement response of *Haliotis* spp is widely published (Morse 1992, Searcy-Bernal *et al.* 1992, Roberts 2001). GABA binds to receptors on the larva resulting

in the arrest of cilia, used for motility, and consequently settlement of larvae on the substratum, whilst at the same time initiating metamorphosis (Morse 1990, Morse 1992, Roberts 2001). However, this response is mediated by the concentration of GABA, which is itself subject to change through degradation by bacteria. Generally, concentrations of GABA between 1×10^{-5} M and 1×10^{-6} M have been shown to induce metamorphosis of abalone larvae, although different species respond differently and higher concentrations and variation in other settlement conditions, including the presence of bacteria and the constituency of seawater, can significantly affect the response of larvae and can result in inhibited metamorphosis and growth or high rates of mortality (Morse 1992, Roberts 2001). The use of antibiotics to reduce bacterial growth and hence support the activity of GABA to induce larval settlement has been demonstrated and applied at commercial scales (Morse 1990, Searcy-Bernal *et al.* 1992). However, reduced bacterial abundance (Anguiano-Beltran & Searcy-Bernal 2007) and bacterial presence (Roberts *et al.* 2007a) can result in reduced post-larval growth and reduced metamorphic response of abalone larvae to a range of benthic diatoms, respectively. Further, the use of antibiotics may cause development of antibiotic resistant pathogens infecting abalone in a hatchery environment as well as having potentially negative effects on bacterial communities on natural reefs, where antibiotics from a hatchery may be introduced (Anguiano-Beltran & Searcy-Bernal 2007).

The addition of GABA (1×10^{-6} M) elicited a significant effect on the proportion of larvae that settled, with greater proportions of larvae settling with the addition of GABA in experiments in this chapter. However, this response was not consistent among all experiments. Despite this inconsistency, the general effect of GABA was small compared to the other factors investigated. It is possible that the concentrations of GABA used were inadequate to elicit a strong settlement response in *H. rubra* or that the concentrations used were significantly affected by the presence of bacteria, as no antibiotics were used in any of the experimental protocols to reduce the likelihood of introducing antibiotics to natural reef, through the flow through seawater system used in the hatchery, which would have aided to reduce bacterial degradation of GABA. Where significant effects occurred, higher rates of settlement over short periods of time (i.e. 30-60 sec.) were associated with higher concentrations of GABA in each experiment. The effect of GABA to increase the proportion of larvae that settled, particularly over short periods of time, along with the high rates of metamorphosis and positive rates of growth shown over 24 and 48 hours in the experiments done in this chapter, and in addition to overwhelming published literature describing the positive affects of GABA on abalone settlement (Morse *et al.* 1979, Moss & Tong 1992b, Searcy-Bernal *et al.* 1992, Searcy Bernal & Anguiano Beltran 1998, Roberts 2001), provided substantial support for the use of GABA (1×10^{-6} M) in the protocol associated with the release of larvae to coastal reefs, described in Chapters 4, 5 and 6.

2.4.2.2. Refrigeration

The use of cool storage to transport abalone larvae from a hatchery to a field release location has been described (Tegner *et al.* 1986, Schiel 1992, Preece *et al.* 1997). However, no assessment of the possible affects of this treatment on the settlement or post-settlement responses of larvae has been discussed. While experiments have described and quantified the effects of delayed metamorphosis of *Haliotis* spp, the subset of experiments described here to investigate the settlement response of *H. rubra* to the effects of refrigeration do not attempt to describe effects over extended periods of time (see Roberts & Lapworth 2001). Rather, this treatment was investigated as a means of storing larvae for the short periods of time required for their transport to the field (24-48 hr.), without the use of antibiotics, the use of which is common in the literature when holding larvae for extended periods of time (Roberts & Lapworth 2001) to minimise bacterial infection and subsequent larval mortality.

Despite significant effects of treatments in combination with that of refrigeration on the settlement response of larvae described here, the general responses of larvae did not indicate that refrigeration alone has a significant detrimental affect on their ability to settle, metamorphose and grow. In fact, results from experiments 7 and 8 indicated that refrigeration may increase the rate at which larvae settle over short periods of time. Further, results from experiment 8 also indicate that rates of metamorphosis are not significantly affected by refrigeration. However, the refrigeration of larvae may have an effect on the rate of growth of newly settled juveniles, at least within the first 48 hours after settlement. The longer term implications of refrigerated storage of larvae on subsequent growth may include increased mortality of slower growing individuals due to higher levels of predation by a known host of predators on smaller juveniles (Shepherd & Turner 1985, Shepherd & Breen 1992, Shepherd & Daume 1996, Naylor & McShane 1997). Further, the apparent reduced rate of growth of settled larvae due to refrigeration may indicate more fundamental effects on the physical, biochemical or neurological development of the settled larvae, which may have significant consequences on their survival, beyond those related to their size (as discussed in Roberts & Lapworth 2001).

2.4.2.3. Temperature shock

The effect of constant temperature on the settlement, survival and growth of *Haliotis* spp has been described in the literature, where the primary aim has been to determine favourable conditions with which to rear larvae in controlled laboratory or hatchery conditions (e.g. Leighton 1972, Ebert & Houk 1984, Hahn 1989b). No literature has described the settlement response of *Haliotis* spp to an abrupt change in temperature prior to settlement, as may be experienced either when transferring larvae from cool storage to ambient temperature prior to release (Tegner *et al.* 1986, Schiel 1992, Preece *et al.* 1997) or, as observed in this thesis, where

larvae are transported directly from the hatchery or from refrigerated storage to field release locations, as described in Chapters 4, 5 and 6.

Results from experiments 9 and 10 supported the hypothesis that a temperature shock, similar to that experienced when transporting larvae from a hatchery to a field location, has no significant effect on the settlement response of larvae, with this response being consistent over periods of time ranging from 60 seconds through to 96 hours and also when exposed to water flow across the settlement substrata. The effect of temperature shock on the metamorphosis and size of larvae was not as conclusive. The proportion of settled larvae that metamorphosed, and their size, after exposure to a 0^{o}C, 5^{o}C or 10^{o}C temperature shock supported the hypothesis that temperature shock does not significantly affect metamorphosis, but not that there was no effect on growth. The insignificant effect of these temperature shocks on the settlement response of larvae and the general response of larvae to still be capable of metamorphosis and growth, despite the variability in results presented in this chapter, provide reassurance of their resilience to withstand similar treatments of these factors when released to field locations.

2.4.3. Factors imposed following release

2.4.3.1. Exposure time and water flow

The time taken by abalone larvae to settle, remain on the substratum and metamorphose can have significant consequences on their dispersal, the likelihood of finding appropriate habitat to settle (McShane 1992, 1995), and their subsequent survival and growth (Searcy-Bernal 1999, Roberts & Lapworth 2001). The effect of water flow on the dispersal of *Haliotis* spp has been described for a number of species. Much of this literature has discussed physical attributes of the fertilised egg and behavioural attributes of larvae, in combination with local hydrodynamic forcing, to describe patterns of recruitment (Prince *et al.* 1987, McShane *et al.* 1988a, Prince *et al.* 1988a, Tegner 1992, Sasaki & Shepherd 1996). Moreover, water flow has been hypothesised to significantly effect the retention of released abalone larvae to field locations, where the release of larvae with and without protection from water flow (and additional factors, such as predatory fishes) has been used to demonstrate its effect in the field (Schiel 1992, Preece *et al.* 1997).

Results from experiments 8 - 10 support the hypotheses that the settlement response of larvae is significantly affected by the time they are exposed to the settlement substrata and that this response is consistent among factors of water flow, refrigerated storage and temperature shock. Similarly, results from experiments 2 and 10 demonstrated that greater proportions of larvae settle in the absence of water flow across the settlement substrata and generally more than double the proportion of larvae settle in the absence of water flow during the first 30 minutes of

exposure to the settlement substrata. Results from experiment 8 also supported the hypotheses that exposure time does affect the proportion of settled larvae that metamorphose where as differences in water flow has little influence.

There is no literature describing the very short-term settlement response of larvae to factors, including water flow, in the series of experiments presented here. It is over the short time scales investigated in this series of experiments (less than 30 min.) that larvae released to field locations may be transported away from appropriate habitats for settlement and beyond areas defined for their release and subsequent assessment of survival and growth. In light of this, identification of factors likely to reduce the time larvae spend in the water column was essential to the success of subsequent field releases, as described in Chapters 4, 5 and 6.

2.4.4. Contributing factor - Batch

Differences in the settlement response of haliotid larvae among different batches has been reported by other authors (Baloun & Morse 1984, Trapido-Rosenthal & Morse 1986, Roberts & Nicholson 1997). Further, a number of endogenous and exogenous mechanisms may contribute to the variation in the settlement response observed among batches, including variation in egg quality (Daume & Ryan 2004, Huchette *et al.* 2004) and variation in benthic diatom cover during experimental substrata conditioning in filtered seawater. Further, the interaction between these factors and others may also change with the age of the larvae, as rate of larval settlement in response to cues has been documented to be affected by larval age (Moss & Tong 1992a).

Despite significant variation in the settlement response of 7 and 8 day old *H. rubra* larvae among batches through time described in experiments 11 and 12, the settlement response between times tended to describe the greatest differences. This variation in the settlement response among batches had a relatively low effect, as reported in other studies (Roberts & Nicholson 1997, Roberts 2001).

2.4.5. Conclusion and recommendations

It is clear from the experiments completed in this chapter that several factors including the type of substratum, those related to handling and transport of larvae from a hatchery to a field release location and those experienced immediately at the point of release can affect the ability of *H. rubra* larvae to successfully settle, metamorphose and grow. Perhaps most importantly, the low rates of settlement over short time periods in the presence of relatively low water flow (i.e. ~20% over 30-60 sec.), suggest settlement within release locations will be limited unless larvae can be positioned in areas that maximise the duration of their exposure to appropriate habitat. Appropriate habitats in the field are generally discrete and restricted, being interspersed with areas where successful settlement and survival is likely to be low. These results along with

observations from field locations emphasise the importance of investigating release methods such as the larval deployment pump and physical shelters (Chapter 4), which allows the controlled delivery of larvae into niche habitats with reduced water movement.

The standard protocol for the handling, transport and release of developed larvae to coastal reefs, as supported by the experiments presented here should include: 1) limiting the time larvae are held in cool storage (≤ 24 hr), for transport prior to release; 2) buffering larvae from significant temperature shock while being transported; 3) treatment of larvae with GABA at a concentration of 1 x 10^{-6} M prior to release; and 4) the release of larvae to targeted niche habitats that offer potential settlement substrata, such as a significant cover of CCA, that are exposed to relatively low levels of water flow, such as among the interstitial spaces on a reef. These niche habitats are also likely to provide an environment where larvae will be exposed to a settlement substrata for relatively longer periods of time, and are therefore likely to increase the proportion of released larvae that settle.

The factors related to the handling and transport of developed larvae investigated in these experiments had relatively minor effects on the successful settlement of larvae. Indeed some combinations of treatments, expected to compromise the ability of larvae to settle, resulted in increased rates of settlement. Regardless, the results emphasise the resistance of *H. rubra* larvae to the handling and transport techniques developed to release them to field locations to assess their settlement, survival and growth as described in Chapters 4, 5 and 6.

3. BATCH-TAGGING *HALIOTIS RUBRA* LARVAE AND JUVENILES

3.1. INTRODUCTION

Measurements of the success of releasing animals from hatcheries into wild populations are difficult as reared individuals must be recognized from wild stocks (Blankenship & Leber 1995, Hilborn 1998, Caddy & Defeo 2003, Bell *et al.* 2005). Indeed, the success or failure of a number of stock enhancement programs have been difficult to quantify due to the inability to identify released individuals or their progeny. This inability to measure the success of enhancing wild populations has raised concern over the continued investment of resources into such programs (Hilborn 1998, Bell *et al.* 2005).

The identification of recaptured individuals, released from a hatchery, and unambiguous differentiation from wild conspecifics requires effective tagging. Tagged individuals allow estimates of survival and other demographic attributes, such as growth, to be determined from recaptured individuals. Tagging methods can be defined within four broad categories: physical, chemical, electronic and biological tags (Bell *et al.* 2005). While alternate categories have been described (Rothlisberg & Preston 1992, Thorrold *et al.* 2002), the specific methods used for tagging individuals remain the same (Rothlisberg & Preston 1992, Thorrold *et al.* 2002, Bell *et al.* 2005). Physical tags can include identifying labels that are fixed to, or inserted through, external structures. Chemical tagging can range from stable isotope analysis to immersion in, or injection of, fluorescent compounds (usually antibiotics). Electronic tags can be as simple as implanting a passive integrated transponder (PIT) tag, to the use of more expensive pop-up satellite archival tags. Biological tags can range from inheritable genetic markers and geographically distinct parasites to external physical markings, either natural or imposed from diet, branding or removal of some physical structure from an individual e.g. tail or fin clipping.

A range of attributes are required to identify individuals and determine the success of a stock enhancement program (Rothlisberg & Preston 1992). The tag must be able to: 1) mark early life history stages; 2) be detectable in subsequent life history stages; 3) be unique to the local population; 4) be harmless to the tagged animal and any consumer and be acceptable to the public; 5) be inexpensive to mark and detect; 6) allow rapid marking and detection; 7) allow for multiple marking; and 8) be transmitted to subsequent generations.

Although all of these attributes are highly desirable for any tag, only a sub-set of these attributes may be needed for some objectives (see Rothlisberg & Preston 1992, Bell *et al.* 2005). For example, evaluating the contribution of released individuals to subsequent generations requires a heritable tag, but this is unnecessary where the objective of the study is to determine the survival of released individuals to a local population.

Many tagging methods focus on identification of recaptured individuals and several have proven to be successful at identifying recaptured invertebrates (see Nielson 1992, Bell *et al.* 2005). However, tagging to uniquely identify individuals can often be difficult, due to extensive handling and there are logistic difficulties if large numbers of individuals are required to be tagged. The latter is particularly relevant where individuals are released to investigate the efficacy of enhancing local populations.

Batch-tagging is an efficient method for tagging large numbers of individuals with a common tag, or mark, that is added simultaneously to all individuals within a group. Batch-tagging can also facilitate measurement of an individual's growth, if the tag can be; 1) incorporated into a structure within the individual that regularly changes size through time; 2) where the tag or mark does not affect growth, or the affect can be accounted for; and 3) where the tag persists in the individual through to its recapture.

Methods for batch-tagging marine invertebrate larvae or very young juveniles have been established (see reviews by Levin 1990, Thorrold *et al.* 2002). In particular, the use of a fluorochrome dye, calcein, in immersion experiments, to batch-tag a range of invertebrate larvae (Rowley & Mackinnon 1995) including molluscs (Moran 2000, Moran & Marko 2005) has been effective. Fluorochrome dyes, such as calcein, are incorporated into calcified tissues, such as shell or bone, as they are deposited, providing a mark that is persistent and visible in the structure with the aid of an appropriate filtered light source (Day *et al.* 1995, Rowley & Mackinnon 1995, Collin & Voltzow 1998, Moran & Marko 2005). However, a number of studies have identified, or inferred, reduced survivorship and/or growth, associated with tagging using calcein, particularly in fish species (Brooks *et al.* 1994, Bumguardner & King 1996), but also invertebrates (Thebault *et al.* 2006).

Calcein has been used to tag the shell of larval abalone (*H. kamtschatkana*) and it has been demonstrated that the mark is retained within the shell during settlement and metamorphosis. The mark remains imbedded in the growing shell, positioned at the top of the spire (Collin & Voltzow 1998). This, therefore, provides a means of positively identifying recaptured individuals that were tagged and released as larvae. There are no data describing the success of tagging larval or early juvenile *H. rubra*. Groups of juvenile or adult abalone have been batch-tagged either through a diet of varying alga (as observed by Leighton 1961, Olsen 1968, Hahn 1989c, Schiel 1993), artificial feed (Gallardo *et al.* 2003) or immersion in, or injection of, fluorochrome dyes (Pirker & Schiel 1993, Day *et al.* 1995). The development of specific methods to batch-tag abalone, fed commercial diets, is generally unnecessary because the commercial diet itself results in an indelible tag, in the form of a distinct blue-green colouration of the shell (Gallardo *et al.* 2003). This blue-green coloured shell, remains in the centre of the spire of the shell as the individual grows, with new shell deposition coloured the common,

natural red-brown once a more natural algal diet is consumed (Gallardo *et al.* 2003), such as upon release to natural reefs.

The objective of this chapter was to develop and test methods that enabled batches of larval and juvenile *H. rubra* to be reliably and consistently tagged and subsequently identified on natural reefs over periods of time ranging from 1 day to >2 years. This was a necessary prerequisite before larvae and juveniles were released to natural reefs, as described in Chapters 4, 5 and 6.

3.2. MATERIALS AND METHODS

3.2.1. Batch-tagging larvae

Experiment 1

A laboratory experiment was done to determine: 1) the combination of concentration and immersion time that would provide an indelible mark that could be detected in hatchery-reared juveniles after six months; and 2) if calcein could be used to batch-tag *H. rubra* larvae without substantially affecting their survival and growth. The survival of settled larvae and subsequent juveniles was observed and the shell length of juveniles from treatments of the highest concentration and longest immersion time were measured. The persistence and level of epi-fluorescence was measured from the larval shell that became embedded in the spire of each juvenile. It was hypothesised that the levels of epi-fluorescence would be greater at higher concentrations and for longer immersion times. Further, it was predicted that there would be no substantial affect of batch-tagging larvae with calcein, on their growth as juveniles.

Developed larvae were added, at a concentration of about 100 ml^{-1}, to one of two larval rearing tanks (180 L), containing calcein (Sigma-Aldrich, Product No. C8075) at a concentration of either 0.05 or 0.1 $g.L^{-1}$ in sodium bicarbonate (0.1 $g.L^{-1}$) buffered seawater. The temperature of the seawater in the tanks was maintained at 19°C (± 0.5°C). Larvae were held in each tank for 24 and 48 hours, with aeration and without water flow.

After 24 and 48 hours, approximately 3 000 larvae from each treatment tank were removed. These 4 groups of larvae were placed into separate, 14 L settlement tanks. Each settlement tank contained twelve poly-vinyl-carbonate (PVC) sheets (150 x 200 mm), stacked on their edge and separated by about 30 mm. These sheets had been conditioned in aerated seawater for ~4 weeks with each covered by an algal bio-film able to support abalone larval settlement (*sensu* plates) (Ebert & Houk 1984, Hahn 1989c). Each tank had a constant flow of fresh seawater at ambient temperature (~19°C). Settled larvae were grown on the plates until food was limiting (~75 days). They were then removed from the plates and returned to the 14 L tanks and fed a commercial abalone diet for the term of the experiment. Replicate samples of twenty individuals

were haphazardly sampled from each treatment combination and stored 15, 23, 30, 37, 56, 75, 97, 120 and 260 days after settlement. Each sample was preserved in 70% alcohol and stored in the dark. The spire of each shell, in all replicate samples, was observed under a compound microscope with illumination via a 50 watt, reflected, ultra-violet light source through a blue-light (I2) filter block. This allowed observation of the calcein epi-fluorescence bound in the larval shell.

The level of epi-fluorescence was scored on a graduated, subjective visual index from 0 to 4. A score of 0 indicated no discernable epi-fluorescence and no positive identification of a tagged larval shell in the spire of the juvenile shell, whilst a score of 4 indicated a bright and obvious mark with clear definition of the larval shell in the spire of the juvenile shell. Scores of 2 and above indicated unambiguous positive identification of calcein in the larval shell.

The effects of the factors calcein concentration (0.05 and 0.1 $g.L^{-1}$), immersion time (24 and 48 hr.) and time (15, 23, 30, 37, 56, 75, 97, 120 and 260 days post-settlement) were analysed using the data of the subjective scores of epi-fluorescence. Cochran's *C*-test was used to determine the homogeneity of variances (Underwood 1997). Analysis of variance (ANOVA) was used to test for differences among treatments.

The maximum shell length of each individual, from all samples taken from the treatment with the highest concentration and longest soak time, was measured. These data were compared with predicted lengths of juveniles of the same age, calculated from minimum and maximum daily growth rates reported for batches of abalone reared in the same hatchery facility and treated similarly, without having been exposed to a batch-tagging treatment as larvae. The rates of growth of abalone reared in the hatchery from settlement through to removal from plates and not batch-tagged as larvae, ranged from 24 $\mu m.day^{-1}$ to 50 $\mu m.day^{-1}$. After removal from plates and fed a commercial diet the length of juveniles increased linearly at rates that ranged from 50 $\mu m.day^{-1}$ to 100 $\mu m.day^{-1}$ for the following year, although rates of between 60 $\mu m.day^{-1}$ and 75 $\mu m.day^{-1}$ were most common (after Heasman *et al.* 2004).

In addition to experiment one, juveniles (n = 9), sampled from field locations where batch-tagged larvae (using the standard release protocol described below, i.e. calcein at 0.05 $g.L^{-1}$ for 48 hrs) had been released 553 days previously as part of experiments described in Chapter 6, were collected. Any fouling on the spire of each shell was carefully removed using dissecting tools whilst viewing the shell under a dissecting microscope. The spire of the shell was examined for the presence of a calcein labelled larval shell (Plate 3.1), as described in the methods for experiment one and using NIH image analysis software, described in Chapter 2. Results presented in this chapter from recaptured abalone released as larvae, were used for

determining the success of the batch-tagging method only. Experimental results regarding rates of survival and growth of released larvae are reported in Chapter 6.

3.2.2. Batch-tagging juveniles

All juveniles used in experiments described in this thesis were settled as larvae on plates and grown until reaching a length of about 1.5 mm. These early juveniles were removed from settlement plates, held in flow-through seawater tanks, and fed a commercial abalone diet. The commercial diet fed to juveniles resulted in the blue-green colouration of the shell (see tagged shell on individuals in Plates 3.2 and 3.3). This shell colour is distinctly different from that of the natural, red-brown, shell colour of wild conspecifics. This diet-induced colouration has not been observed in natural populations of *H. rubra* in over 14 years of quantitative surveys of these populations, in both New South Wales and South Australia (personal observation) or among wild populations of abalone observed throughout the world (S. Shepherd pers. comm.). As a consequence, no formal experiments were conducted to further investigate a means of batch-tagging juveniles to differentiate them, once released, from wild conspecifics.

The relationship between the maximum length of abalone and, hence, the size of the tag at the time of release, was described for batches of juveniles reared in the hatchery over the size range (2-15 mm) of juveniles commonly used in experiments described in Chapters 4, 5 and 6.

Abalone reared in the hatchery and released as juveniles, as part of experiments reported and discussed in Chapter 6, were collected when recaptured and the presence and persistence of the blue-green shell was assessed to support the use of this method to batch-tagging juveniles. It was predicted that the colouration of the hatchery-reared juvenile shell would persist in the spire of recaptured individuals. Further, it was predicted that the colouration of newly deposited shell would change to a natural red-brown, enabling the individual size at release of recaptured abalone to be determined (length of blue-green shell), in addition to growth (red-brown shell) subsequent to release. Observation of the blue-green shell and the delineation between this and the subsequent red-brown shell in some cases required the removal of natural fouling.

3.3. RESULTS

3.3.1. Batch-tagging larvae

Experiment 1

The calcein tagged larval shell was visible in the spire of all abalone shells from all treatments investigated in experiment one (Figure 3.1). Higher and more consistent levels of epi-fluorescence were observed in the tagged larval shell in the spire of juveniles where larvae were immersed for 48 hours, irrespective of the concentration of calcein (Figure 3.1). However, the rank order of levels of epi-fluorescence among concentrations of calcein varied within immersion times, resulting in a significant interaction (Table 3.1). There was no significant difference in the level of epi-fluorescence associated with the time (e.g. loss of epi-fluorescence post-settlement) that abalone were sampled (Figure 3.1; Table 3.1). The larval shell could be easily distinguished in ≥85% of the abalone treated at the lowest concentration of calcein (0.05 $g.L^{-1}$) for the shortest immersion time (24 hr). However, within these treatments, within any one sampling time, up to 15% of samples were faint, providing an ambiguous identification of the calcein tagged larval shell and attaining a score of 1.

The mean length of juveniles, tagged as larvae in a calcein concentration of 0.1 $g.L^{-1}$ for 48 hours (i.e. the highest concentration; longest immersion time), was within the bounds reported for batches of juveniles reared in the hatchery to the same age (25-50 $\mu m.day^{-1}$ on plates and 50-100 $\mu m.day^{-1}$ on a commercial diet) and not subject to batch-tagging as larvae (Figure 3.2). However, the mean length of juveniles sampled 75, 97, 120 days post-tagging indicate the growth rate during this time was close to the lower range of that predicted from juveniles not batch-tagged as larvae.

A total of nine *H. rubra* were recovered in samples taken within two of three locations where tagged larvae were released 553 days previously (sampling at one location detected no abalone). Five of the nine individuals were positively identified as having been released as tagged larvae, with the unambiguous tag of the epi-fluorescing calcein labelled larval shell in their spire (Plate 3.1).

3.3.2. Batch-tagging juveniles

There was a strong relationship between the size of the juvenile shell and the time abalone were reared in the hatchery prior to release (Figure 3.3). Thus, the size of the juvenile shell and hence the biological tag, at the time of release was a function of the rate of growth of juveniles held in the hatchery.

Juveniles that had been reared in the hatchery and fed a commercial abalone diet were clearly identifiable from wild conspecifics, through the distinctive blue-green shell (Plate 3.2). The colour of further shell deposition of hatchery-reared juveniles reverted to the red/brown natural colour within days of release (personal observation). The distinct blue-green spire was obvious in juveniles recaptured after 769 (Plate 3.2) and 777 days, with removal of any fouling of the shell (Plate 3.3).

A. B.

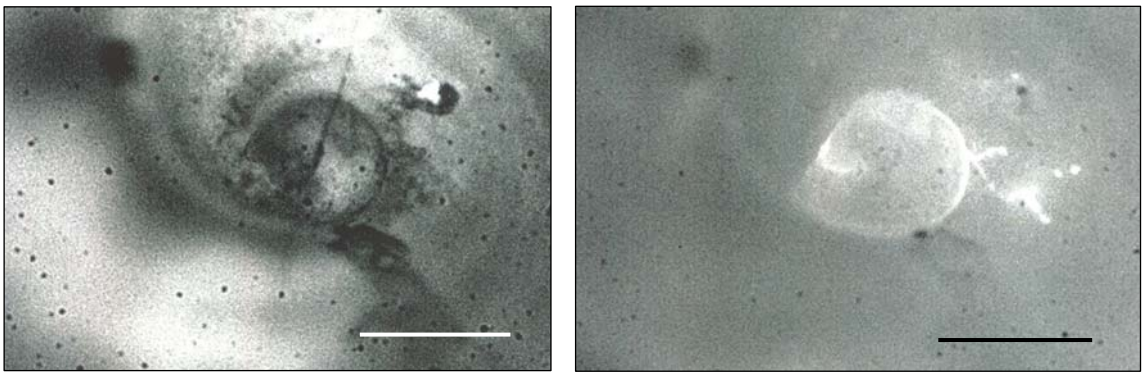


Plate 3.1 Photomicrographs of the spire of an abalone shell, recaptured 553 days after release as a larvae, under transmitted standard light (A) and via a filtered light source, showing the calcein labelled larval shell (B) (400x). Scale bar is 300 μm.

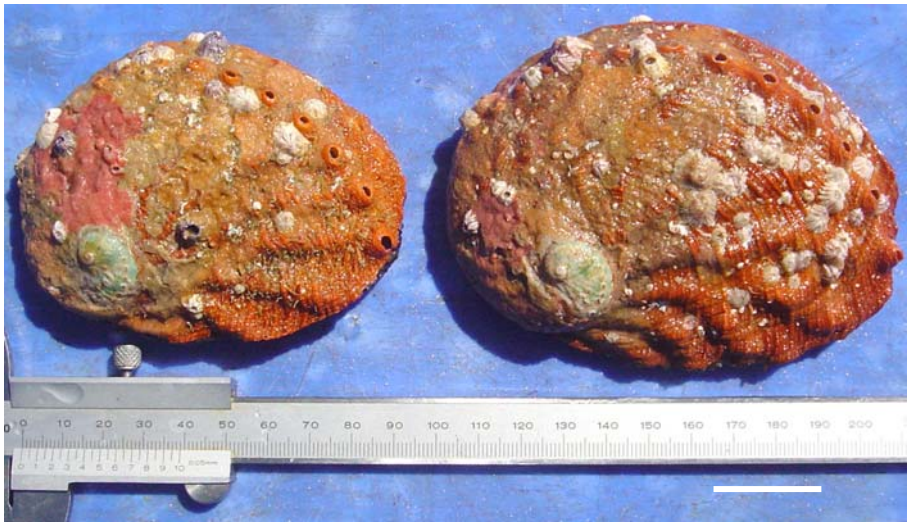


Plate 3.2 Abalone recaptured 769 days after release, showing the distinctive blue-green coloured shell in the spire, indicating the size of the individual at release. Red-brown shell colour is natural shell growth after release. Scale bar is 25 mm.

A. B.

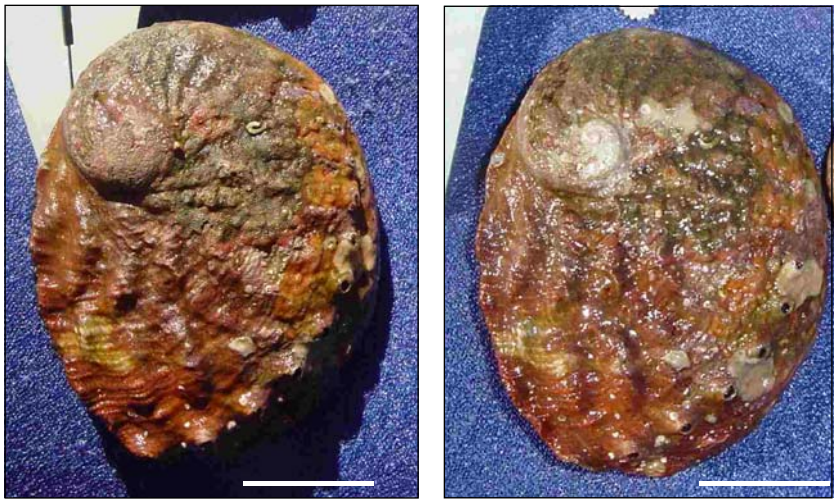


Plate 3.3 An abalone recaptured 777 days after release, showing fouling on the spire of the shell obscuring the batch-tagged shell (A) and the same individual with the fouling removed, showing the distinctive blue-green coloured shell, indicating the size (~12 mm) of this individual at release (B). Scale bar is 25 mm.

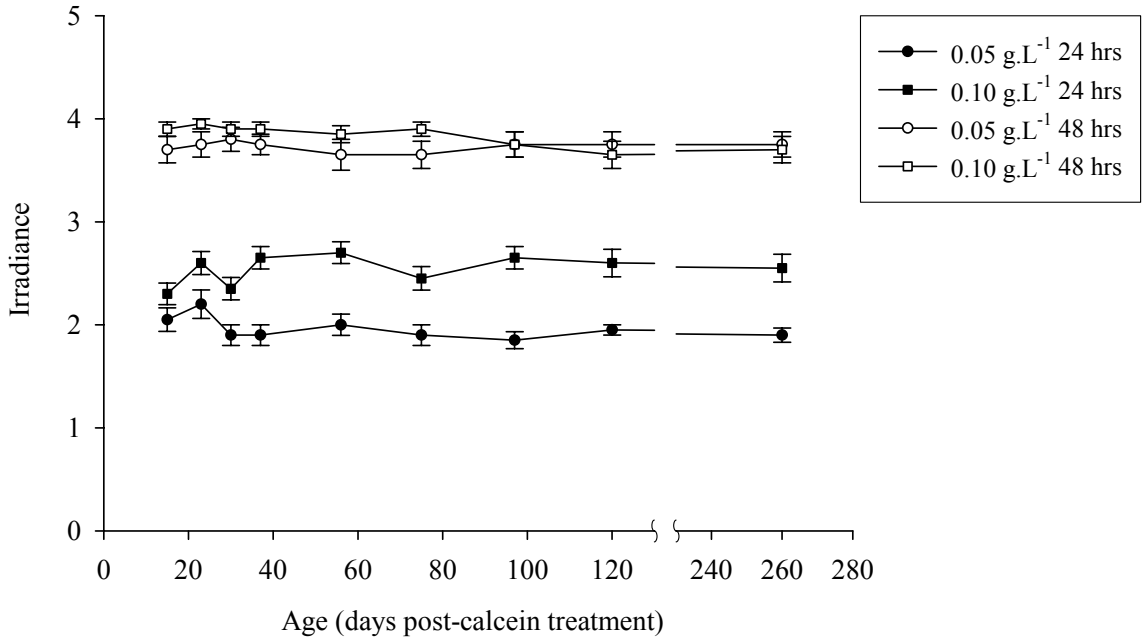


Figure 3.1 Mean level of irradiance (± SE) of the larval shell in the spire of *H. rubra* sampled through time, after larvae were batch-tagged in two concentrations of calcein for two immersion times; $n = 20$.

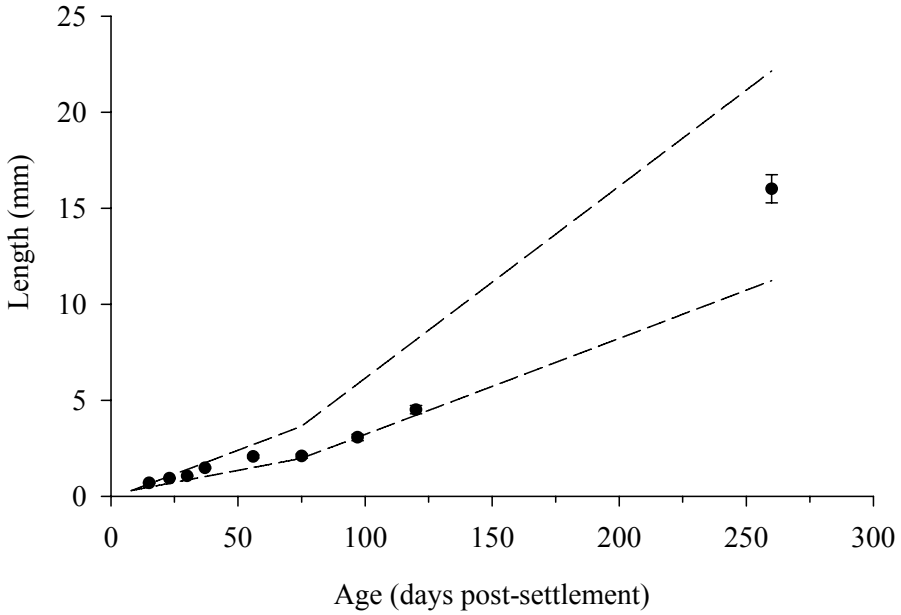


Figure 3.2 Upper and lower predicted lengths (mm) of juveniles reared to the same age and not batch-tagged as larvae, generated from minimum and maximum daily growth rates reported in Heasman *et al*. (2004) (dashed lines; see methods) and mean (± SE) shell length of juveniles, after having been immersed in calcein (0.1 $g.L^{-1}$) for 48 hours as larvae (solid circles); $n = 20$.

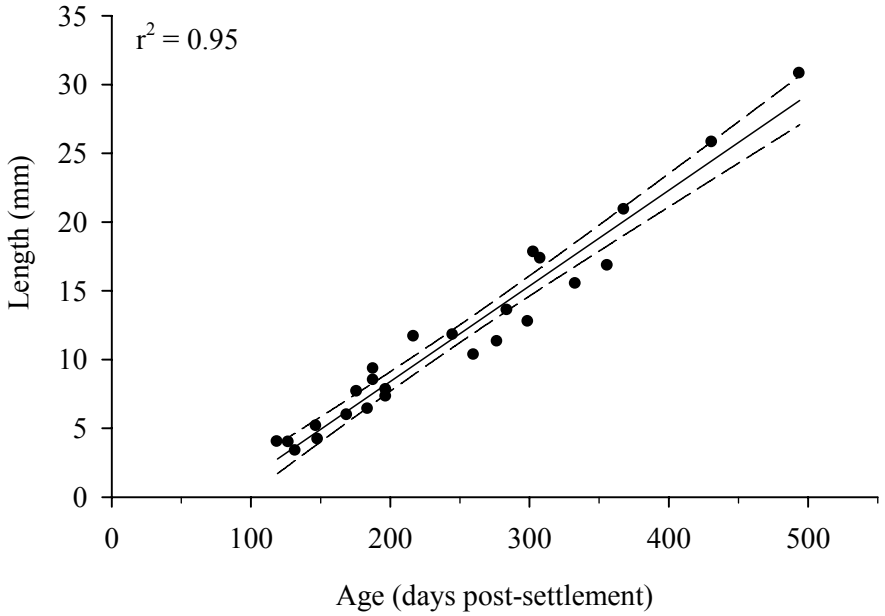


Figure 3.3 Relationship between age and shell length for *H. rubra* reared in the hatchery (n = 100 - 200), adapted from Heasman *et al.* (2004), indicating the size of the juvenile shell, i.e. blue/green coloured tag, at the time of release after settlement. The regression (solid line) and ± 95% confidence interval (dashed lines) are shown.

Table 3.1 Summary of analysis of variance in the level of irradiance of the calcein tagged larval shell in the spire of abalone 15, 23, 30, 37, 56, 75, 97, 120 and 260 days after being treated in two concentrations of calcein (0.05 and 0.1g.L^{-1}) for two immersion times (24 and 48 hrs). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's C = 0.0531, ns; variances 36, df 19).

	Source	df	MS	F	F vs
1	Concentration, C	1	21.01	89.35 **	8
2	Immersion time, I	1	421.67	1793.10 **	8
3	C x I	1	10.03	42.67 **	8
4	Time, T	8	0.20	0.86 ns	8
5	C x T	8	0.14	0.60 ns	8
6	I x T	8	0.22	0.94 ns	8
7	C x I x T	8	0.34	1.46 ns	8
8	Error	684	0.24		
9	Total	719			

3.4. DISCUSSION

An indelible tag enables individuals to be identified and this is fundamental for any investigation where individuals are released and recaptured for demographic studies, including an enhancement program where survival and growth are used to measure success. The objective of this chapter was to develop and test methods that enabled batches of larval and juvenile abalone to be reliably and consistently tagged, to enable their positive identification when recaptured from natural reefs through time. A tagging program, therefore would allow a comprehensive assessment of an ongoing enhancement program (Rothlisberg & Preston 1992, Bell *et al.* 2005).

Simple and reliable methods of batch-tagging *H. rubra* larvae and juveniles were developed and tested. Both enabled released individuals to be positively identified and differentiated from wild conspecifics when recaptured. The methods to batch-tag larvae and juveniles, described in this chapter, addressed a substantial proportion of those attributes desirable for a tag, to identify individuals for a stock enhancement program (Rothlisberg & Preston 1992). I demonstrated that the use of either method to batch-tag larvae or juveniles: 1) enabled the earliest life history stages of *H. rubra* to be tagged; 2) provided a tag that is detectable in subsequent life history stages; 3) allowed released individuals to be differentiated from conspecifics within a local population; 4) was harmless to the tagged individual; 5) was relatively inexpensive; and 6) was relatively quick to tag larvae (48 hr.). Moreover, juveniles are tagged through rearing, with detection of both tags, particularly that of juveniles, being relatively efficient with very little sample preparation time required as whole shells could be used. An additional advantage of these methods is that the non-invasive preparation of samples, for both methods, could be used to identify live animals despite the difficulties of requiring a compound microscope and light source to identify the calcein labelled larval shell.

Data presented in this chapter describe the first investigation and test of a method to successfully batch-tag *H. rubra* larvae, based on published data to date. The persistence of the calcein tagged larval shell in the spire of juveniles was demonstrated by unambiguous identification of the calcein tag in >95% of abalone treated in this experiment and the absence of a substantial difference in the level of epi-fluorescence over time, for up to 260 days post-settlement. Nevertheless, the higher levels of epi-fluorescence observed in the treatment where larvae were immersed for 48 hours combined with the negligible difference between the level of epi-fluorescence among the different calcein concentrations within this treatment, supported batch-tagging of larvae at a concentration of 0.05 $g.L^{-1}$ for 48 hours as a standard protocol, applied to all batches of larvae that were to be released in field experiments described in this thesis.

The use of this protocol was also supported by observations that the treatment of larvae with calcein did not affect larval survival or settlement, and data indicating that there was no substantial difference in the long-term growth of juveniles, after having been tagged at 0.1 $g.L^{-1}$ for 48 hours. However, mean lengths of juveniles within approximately 100 days after removal from settlement plates did indicate the potential for this procedure to effect initial rates of growth, as mean lengths were at the lower range predicted from early juveniles reared in the same hatchery, but not batch-tagged as larvae. Despite this, the mean shell lengths and rates of growth described for the juveniles batch-tagged as larvae and reared in the hatchery were similar to those described of many other haliotids over similar times (see review Kawamura *et al.* 1998), and towards the higher range of those described for *H. rubra* by Daume *et al.* (2000). The positive identification of juveniles recaptured from natural reefs, after being released as batch-tagged larvae 553 days previously, also provides substantial support to the value of this batch-tagging protocol and elicits confidence in its application.

However, it is likely that a proportion of recaptured juveniles, tagged as larvae would not be positively identified. For example, if the larval shell had been physically worn from the spire of juveniles though the course of their life history, or that fouling of the shell or removal of fouling during sampling, damaged the spire then the level of detection would be reduced. There is also some evidence that the intensity of a calcein tag can degrade through time (Leips *et al.* 2001) and when exposed to sunlight (Bashey 2004). Results from the work described in this chapter does not indicate any reduction in the intensity of the calcein tag in juveniles, even after rearing in shallow tanks and exposure to indirect sunlight during 260 days post-settlement. Reductions in the ability to detect a tag will result in an underestimation of the number of individuals surviving from their release as larvae. As a consequence, results from recaptures, identified through the observation of the calcein tagged larval shell, will provide estimates of a minimum number of individuals surviving through time. The extent to which these scenarios would affect true estimates of survival were not examined in this thesis. The double tagging or use of multiple tagging methods of juveniles that have been batch-tagged as larvae, prior to release, would help clarify the extent of possibly under-estimating the survival of released individuals as measured by the identification of a calcein labelled larval shell in the spire of recaptured individuals alone (Treble *et al.* 1993, Barrowman & Myers 1996). Despite the acknowledgement of these limitations the results from this experiment add significant support to the growing body of evidence regarding the value of successfully tagging larvae for identification in stock enhancement programs and other ecological investigations regarding their distribution and abundance (see review Thorrold *et al.* 2002, Moran & Marko 2005).

The need to develop a specific method to reliably batch-tag juveniles, independent of regularly feeding them an artificial diet, was unnecessary. This was because *H. rubra*, fed an artificial diet

from the point of removal from plates (~1.5 mm) to their release, resulted in the development of a distinct blue-green coloured shell that readily distinguished these juveniles from wild conspecifics during all future stages of their life history. Other significant advantages of this method included the ability to positively identify individuals in the field and underwater, and it enabled large numbers of even very small juveniles (<5 mm) to be tagged without their excessive handling, and hence avoided potential subsequent high rates of mortality.

There was strong anecdotal evidence from short-term recaptures (<1 month) at multiple locations, that the colouration of the shell of released juveniles deposited post-release rapidly changed from blue-green to a natural red-brown. There was also little evidence of the blue-green shell eroding or becoming faint, and thus preventing positive identification of hatchery released and recaptured abalone through time. The use of measurements of the blue-green shell in the spire of recaptured abalone to indicate the individual length at release was supported by the persistence of the blue-green colouration and subsequent change in colouration of further shell deposition following release. Consequently, this approach provides a simple means to measure individual growth rates of released juveniles, and for these measures to be made in the field and underwater with the use of vernier calipers.

Despite the significant advantages of this method to batch-tag juveniles it is possible that the development of this conspicuous tag (shell colour) could reduce predator avoidance and increase susceptibility to visual predators (Catalano *et al.* 2001), both of which could have significant implications on the use of this method to enhance local abalone populations. However, it is likely that the effect of this distinctive identification on the survival of juveniles could be reduced if they are released to areas of reef where their exposure to visual predators is limited until they are able to disperse to more cryptic habitats (Tegner & Butler 1985, McCormick *et al.* 1994, Shepherd *et al.* 2000). It is also possible that such conspicuous colouration could be minimised by supplementing artificial diets with natural food sources or with specific nutritional additions to the diet so that a conspicuous single band of shell colour or less conspicuous whole shell could be used to tag individuals.

An additional difficulty of batch-tagging is that it does not allow for the identification of individuals that have been recaptured multiple times and does not, therefore, support the determination of any sampling efficiency estimates (Dixon *et al.* 2006). For example, recapture data from batch-tagging cannot demonstrate the survival of individuals that were not observed during initial sampling times, as is possible with tagging methods that identify each specific recaptured individual. Batch-tagging is therefore unable to provide a scaling parameter to support the reassessment of initial estimates of survival, where some individuals may not have been observed, and therefore were not included in initial estimates of survival.

3.4.1. Conclusion and recommendations

The development of tagging methods and inherent hatchery practices, described in this chapter, provide the first means to successfully batch-tag *H. rubra* larvae and outline the successful batch-tagging of juveniles, as used in all subsequent releases of *H. rubra*, described in this thesis. Large numbers of larvae and juveniles could be reliably and consistently batch-tagged at relatively low cost and in a short amount of time. A standard tagging protocol for the purpose of this thesis was adopted and routinely applied, where batches of larvae released to natural reefs were batch-tagged by immersion in calcein at a concentration of 0.05 $g.L^{-1}$ for 48 hours. All juveniles that were released were subject to a diet consisting of a commercial abalone feed for a period of time long enough to provide an indelible blue-green colour in their shell.

4. HANDLING AND RELEASE OF *HALIOTIS RUBRA* LARVAE AND JUVENILES

4.1. INTRODUCTION

The practical release of hatchery-reared individuals into a marine environment to enhance local populations requires a series of logistical procedures to be undertaken. Typically, individuals reared in a hatchery are required to be removed from a holding tank, their quantity determined and groups of individuals separated into manageable units of equal number, to be stored and transported, prior to release. Stresses associated with handling and transport processes (Berka 1986, Estudillo & Duray 2003, Purcell *et al.* 2006, Lorenzon *et al.* 2007) as well as altered behavioural responses of individuals associated with these processes (van der Meeren 1991, Olla *et al.* 1994, 1998, Kellison *et al.* 2000, Masuda & Ziemann 2003, Le Vay *et al.* 2007) can substantially affect rates of mortality prior to release and contribute significantly to mortality within short periods of time (<60 days) after release.

A number of methods of releasing hatchery-reared abalone larvae to natural reefs have been developed and used. Tong *et al.* (1987) transported *H. iris* larvae in seawater in 20 L buckets and tipped them into shallow, rocky subtidal areas at low tide. Other studies have described the storage and transport of haliotid larvae suspended in seawater prior to their release by a diver (Schiel 1992, Gutierrez-Gonzalez & Perez-Enriquez 2005), directly into the sea from a boat (Gutierrez-Gonzalez & Perez-Enriquez 2005) or on damp mesh screens prior to their re-suspension in seawater and then pumped from containers on a boat down a hose to the reef (Preece *et al.* 1997).

Methods of releasing large numbers of larvae directly from containers does not provide a means to evenly distribute them among a large release area or support their directed release to substrata where greater rates of settlement may be possible. For example, substrata exposed to reduced water flow (Butman *et al.* 1988). Areas of low flow are found among interstitial spaces around boulders and under canopies of foliose alga. Moreover, other niche habitats provide strong cues for settlement, such as substrata covered with crustose coralline alga (CCA) (Shepherd & Daume 1996, Roberts *et al.* 2004, Kawamura 2007). However, the release of many groups of small numbers of larvae from containers, to facilitate their even distribution within a release location is impractical. Schiel (1992) and Preece *et al.* (1997) described the use of shelters to contain and increase the settlement of released haliotid larvae. Preece *et al.* (1997) also described a method of releasing larvae by using a pressurised container and hose to pump larvae in seawater to the reef (*sensu* deployment pump). This method enabled the controlled distribution of large numbers of larvae over large areas of reef compared with that possible from containers opened underwater. The majority of studies have concluded that the release of larvae

is unlikely to be the most effective method to enhance local populations of abalone. However, measures of success have generally not discussed the possible effects of the release method on the competency of larvae to settle or their initial survival. The relatively low costs of producing larvae and the possibility of releasing large numbers over large areas with minimal investment may provide the opportunity to supplement alternative and more economically feasible means of enhancing local populations (see Chapter 6).

An alternative to the release of larvae is the release of juveniles. The survival of hatchery-reared juvenile abalone has been demonstrated to be significantly affected by procedures required to manipulate them from a hatchery (Tegner & Butler 1985, 1989, Schiel 1993, McCormick *et al.* 1994, Shepherd *et al.* 2000). The physical disturbance of an abalone attached to a substratum generally results in the abalone displaying a 'clamp-down' response. This response results in the individual attaching itself more firmly to the substrata and makes its removal difficult, with increased risk of physically damaging its muscular foot leading to increased mortality (Gibson *et al.* 2002). Commercial abalone fishers use an abalone iron (a blunt curved steel blade) to quickly remove individuals before their 'clamp-down' response is effected. The removal of large numbers of juvenile *H. rubra* from rearing tanks in a hatchery using a similar method is impractical and likely to result in the injury of a significant proportion of those individuals removed. A number of methods have been investigated to remove abalone from their substratum, including the use of warm water (Schiel 1993), passive migration encouraged by exposure to ambient light (Sweijd *et al.* 1998) as well as the use of anaesthetics (Hahn 1989a, White *et al.* 1996, Chacon *et al.* 2003). Anaesthesia enables large numbers of individuals to be treated and handled to effect their release, including their removal from rearing tanks and distribution of known quantities into large numbers of relatively small replicate physical structures that facilitate their recovery, storage, transport and release to natural reefs. The use of benzocaine as an effective anaesthetic for haliotids has been described in the literature (Leighton 1989, Aquilina & Roberts 2000, Edwards *et al.* 2000, Chacon *et al.* 2003) and is an accepted practice used by commercial, land-based abalone aquaculture facilities (Hahn 1989a). However, the use of any anaesthetic can result in the mortality of individuals under circumstances including exposure to high concentrations or for prolonged periods of time.

Physiological responses of abalone to handling and release conditions can result in direct mortality or increased stress and mortality within short periods of time (<60 days) after release (Schiel 1993, Shepherd *et al.* 2000, Malham *et al.* 2003). For example, the response of juvenile and adult abalone to handling can manifest as excessive production of mucus (Tegner & Butler 1985, Vosloo & Vosloo 2006) that may result in increased detection by predators once released (Tegner & Butler 1985, Shepherd *et al.* 2000). Behavioural responses can also result in increased levels of mortality though hatchery induced naivety, manifested as the inactivity to

escape or seek refuge from predators (Schiel & Welden 1987, Osumi 1999). Moreover, reduced physical fitness or injury to individuals, caused by handling and transport processes, can result in reduced activity and lead to increased exposure to predators once released or direct mortality as a consequence of the injury, as demonstrated by Gibson *et al.* (2002) for wild caught abalone.

Several studies that have investigated the release of juvenile abalone have emphasised the use of some form of physical shelter to reduce high rates of initial mortality. Kojima (1981) described reduced mortality of transplanted juveniles within enclosures that excluded predators. In contrast, Schiel (1992) demonstrated that the provision of an artificial structure to reduce predation may play a relatively minor role in the total mortality of released hatchery abalone within the first 1-2 months and suggested natural shelter provided adequate protection. Similarly, the importance of the natural physical structure of the reef to provide shelter from predators and reduce short-term mortality of released juvenile abalone is well described (Tegner & Butler 1985, Schiel 1992, McCormick *et al.* 1994, Seki & Taniguchi 2000, Shepherd *et al.* 2000, de Waal & Cook 2001, de Waal 2005, Dixon *et al.* 2006, Roberts *et al.* 2007b). However, rates of mortality of released juveniles are still generally high within short periods of time after release, with significant proportions of this mortality attributed to handling stress and exposure to predators. The development and use of small physical shelters (*sensu* deployment device) that provide a simple, cost effective means to hold and transport abalone from a hatchery, reduce handling and provide temporary shelter to juveniles at a release location has been described (Leighton 1985, Ebert & Ebert 1988, Leighton 1989, McCormick *et al.* 1994, Sweijd *et al.* 1998).

The objective of this chapter was to develop methods to deliver hatchery-reared *H. rubra* larvae and juveniles to natural reefs. A series of experiments were done to investigate a) the effectiveness of a deployment pump to deliver consistent numbers of larvae to the reef; b) the effect of the pumping process on the survival and competency of larvae to settle; c) the effect of providing short-term (24 hr.) shelter to *H. rubra* larvae released to natural reefs using the deployment pump; d) the most appropriate combination of anaesthetic concentration and exposure time to remove juvenile abalone from hatchery tanks; and e) the efficacy of two deployment devices to deliver juvenile abalone from the hatchery to natural reefs.

The development of these handling and release methods was essential to support the release of different life history stages of *H. rubra* in field experiments described in Chapters 5 and 6.

4.2. MATERIALS AND METHODS

A series of 4 laboratory and 3 field experiments were designed to test the methods used to handle and release hatchery-reared larval and juvenile *H. rubra* and provide support for the use of these methods in subsequent releases described in Chapters 5 and 6 (Table 4.1). Laboratory experiments were designed to investigate the effect of a combination of GABA at a concentration of 1 x 10^{-6} M (hereafter referred to as GABA) and the refrigeration (24 hr.) of larvae on the rate of supply and the proportion of larvae that settle after simulated release using a deployment pump (Table 4.1, Experiments 1 and 2). A field experiment was done to investigate the effectiveness of releasing larvae to areas of natural reef within physical shelters (Table 4.1, Experiment 3).

The most effective means of removing juvenile abalone from substratum in the hatchery was investigated using the anaesthetic benzocaine (Ethyl 4-aminobenzoate; Sigma-Aldrich, Product No. E1501) in a controlled laboratory experiment. Treatments were anaesthetic concentration (0.5, 1.0 and 2.0 ml.L^{-1} seawater) and exposure time (5, 10 and 20 min.). The number of juveniles removed and their subsequent recovery, following treatment was measured (Table 4.1, Experiment 4). Finally, field experiments were done to investigate the proportion of juveniles that remained on deployment devices through time (Table 4.1, Experiments 5 and 6). All larval and juvenile abalone released to field locations in this chapter were batch-tagged as described in Chapter 3.

4.2.1. Handling and release of larvae

Larvae were sourced and handled according to the recommendations described in Chapter 2. Where larvae were released they were transported to the release location in a 1 L container(s) inside a thermally insulated container containing an ice brick and acclimated to ambient seawater temperature (~20 mins) prior to their release.

A pilot experiment was done to trial methods to release large numbers of larvae (50 x 10^3) to large areas (>100 m^2) of reef. Groups of ~50 000 larvae were transported in four 1 L plastic containers and four 80 μm mesh bags layered between wet sponge. Larvae were transported directly from the hatchery and one of each group (1 L container and mesh bag) was released by divers on the reef at each of four locations. Practical application and observations of these releases indicated the difficulty in evenly distributing larvae over areas of reef. Larvae generally released as small 'clouds' into the water column immediately above the substratum. These releases also drew aggregations of, and high levels of activity from, predatory fishes surrounding the 'clouds' of larvae. From these observations and the logistic difficulty of

releasing large numbers of small volumes of larvae to multiple areas within a release location the continued application of these specific release methods was deemed impractical.

4.2.1.1. Deployment pump

A pressurised deployment pump was designed and manufactured to enable large numbers of larvae to be delivered from a sealed drum down a length (~20 m) of hose to within close proximity (<150 mm) of the natural reef (Plate 4.1). Larvae were added to the drum containing ~34 L of seawater and the drum sealed. A constant supply of compressed air, sourced from a connected SCUBA bottle, was delivered through an air-stone on the floor of the drum resulting in the mixing of its contents. Regulation of the rate of air delivered into the drum in concert with a pressure relief valve, fitted through a connector in the lid of the drum, enabled enough pressure inside the drum to pump the contents through the hose to the reef (<8 m). The flow of larvae in seawater from the drum was regulated via taps on the drum and at end of the hose and directed onto the substratum by a diver. Trials of this deployment pump indicated that the contents of the drum (35 L seawater) was delivered through the hose in approximately 16 minutes. This rate of flow enabled estimates of the rate at which larvae were delivered to field locations to be made to assist in ensuring their even distribution, assuming their even rate of supply from the deployment pump.

Experiment 1 - Supply of larvae from the deployment pump

Experiment one was designed to investigate the rate of supply of larvae from the deployment pump under control and treatment conditions similar to those proposed for field releases, as recommended for the standard protocol for the release of larvae discussed in Chapter 2. Larvae (7 and 8 days old) were exposed to treatments of GABA (0×10^{-6} M and 1×10^{-6} M) and refrigerated storage (0 and 24 hr.), as described for similar treatments of larvae in Chapter 2 (Table 4.1). The age of larvae was analysed separately as age was not independent.

It was hypothesised that fewer larvae would be delivered in samples from the drum where GABA and/or refrigerated storage was used because these treatments would result in greater larval settlement on the inside of the drum and therefore fewer larvae delivered from it.

For each age of larvae, four groups of 50 000 larvae in 1 L of seawater were exposed to treatments or controls of GABA and refrigerated storage using methods described in Chapter 2. Once treated, each group was added to 34 L of seawater in the drum to provide a final volume of 35 L. The drum was then sealed and pressurised and four replicate 50 ml samples were taken at the start of the hose. Additional 50 ml samples were taken at the end (20 m) of the hose when the drum was full and from the start and end of the hose when the drum was half full and almost empty (~5 L remaining). Samples were passed through a sieve (80 μm) and the contents stored in 70% alcohol. The number of larvae in each replicate was counted using a dissecting

microscope. Samples taken from when the drum was half full and almost empty and those from the end of the hose did not form part of the formal analyses as samples were not independent of those collected at the start of the hose when the drum was full. However, these samples enabled estimates to be made of the proportion of larvae not delivered from the drum.

Experiment 2 - Settlement of larvae delivered from the deployment pump

The process of releasing larvae from the deployment pump exposed larvae to vigorous mixing of the contents in the drum through the introduction of compressed air through an air-stone. It was not known if larvae exposed to the process of delivering them from the deployment pump would affect their competency to settle. Given that the rate of flow generated when the drum was under pressure resulted in the contents being emptied within approximately 16 minutes, larvae being delivered from the drum towards the end of this process would have been exposed to more agitation than those released when the drum was full.

Experiment two was designed to investigate the competency of larvae to settle after being exposed to the pumping process when the drum of the deployment pump was full and almost empty (Table 4.1). It was hypothesised that a greater proportion of larvae would settle when delivered from the drum when it was full compared with when it was almost empty (~5 L), because the vigorous mixing of the contents of the drum would affect the competency of larvae to settle. The experiment was repeated with two independent batches of larvae. Time was analysed separately due to the low degrees of freedom for the factor drum if the analyses were combined. Control treatments were disturbed during the settlement time and could not be used to provide a formal comparison.

Three replicate samples were taken from the drum when it was full and almost empty. Approximately 100 ml was taken from the pump for each sample, to collect ~300 larvae per sample, as similarly used in the settlement experiments done in Chapter 2. Larvae in both batches were treated identically. Within approximately 30 minutes of samples being taken each sample was poured into a replicate of the miniature down-welling system that contained a standard settlement substratum and had a low rate of water flow (see Chapter 2, section 2.2.6). After 24 hours both the substrata and larvae remaining in the down-weller were stored and sampled as described for the standard laboratory procedure in Chapter 2 (see section 2.2.5). In summary, the settlement substratum was removed from the down-weller and larvae remaining in the down-weller were collected (rinse sample). Samples were stored in separate jars, in 70% alcohol and the number of larvae in each sample counted. The proportion of larvae that settled from the sample was calculated from the number of larvae on the substrata divided by the sum of those on the substrata and in the rinse sample.

4.2.1.2. Deployment shelters

Experiment 3 - Deployment shelters

In experiment 3, the effect of providing physical shelter to *H. rubra* larvae released to a field location, via the deployment pump was investigated. It was hypothesised that a greater number of larvae would settle within sub-sites afforded shelter compared with sub-sites without shelter after they had been released for 24 hours. A physical shelter consisted of a canopy (an open based cone with a basal area of 1 m^2) of plastic mesh (~1.5 mm mesh size), weighted with chain around the skirt (Plate 4.2).

To increase the likelihood of detecting settled larvae within the treatments larvae were released at a high density (approximately 22 000.m^{-2}). Approximately 2.2 million larvae were added to the deployment pump and released to a field location that consisted of a natural boulder field covering 100 m^2. Five physical shelters were haphazardly distributed within the location prior to larvae being released. Larvae were released from the hose within 150 mm of the reef. Estimates of the delivery rate of seawater from the pump indicated that approximately equal densities of larvae would be delivered throughout the location including the sheltered sub-sites by pumping larvae into each shelter for a total of 10 seconds, with the remaining larvae evenly delivered throughout the location. After 24 hours shelters were removed and 5 boulders (~300 mm maximum diameter) were collected from within each sheltered sub-site and from within five equivalent, haphazardly located, unsheltered sub-sites. These replicate samples were considered to be independent as boulders were semi-uniformly distributed throughout the experimental area and samples consisted of less than approximately 5% of the available habitat within each Sub-site. The number of boulders within each 1 m^2 sub-site was counted. The number of boulders and the average number of larvae from the five boulders from each sub-site was used to calculate the mean number (+SE) of settled larvae.m^{-2} within each sub-site. Boulders collected by divers were placed in plastic bags and the bags sealed *in situ*. Bags containing boulders were transported to the laboratory between layers of foam in water filled bins to minimise their movement and the possibility of damaging the sample. Within 4 hours of collection each boulder was soaked in 70% alcohol for 5 minutes and brushed to remove all surface material (including settled larvae). Samples were sieved (80 μm) and the particulate matter stored in 70% alcohol. The sieved material was sorted under a dissecting microscope and the number of larvae counted. A sub-sample (minimum 30%) of all abalone larvae sorted was examined to ensure only calcein tagged larvae were present in samples.

4.2.2. Handling and release of juveniles

4.2.2.1. Anaesthesia

The anaesthesia of groups of juvenile abalone is essential to remove them from the substratum to which they are attached in the hatchery and enable their quantitative assessment (counting and weighing) and manipulation into manageable units for transport and release. A pilot experiment was done to investigate the effectiveness of using benzocaine to remove juvenile *H. rubra* from a standard settlement substratum in the hatchery. A working solution of benzocaine was made (1 g 10 ml^{-1} benzocaine in ethanol; hereafter called benzocaine) to aid its dissolution in seawater. About 100 juveniles (~2.5 mm SL) were exposed to benzocaine at a concentration of 1.0 $ml.L^{-1}$ in seawater (after Edwards *et al.* 2000) for 5, 10 and 15 minutes. Results indicated that this method could effectively remove 20%, 83% and 97% of juveniles at each exposure time respectively, and that ~95% of juveniles had recovered within 18 hours.

Experiment 4 - Removal and recovery of juveniles

Experiment four was designed to investigate if different concentrations of benzocaine and the time juveniles were exposed to these concentrations could be used to reduce the time taken to anaesthetise juveniles and remove them from their substrata in the hatchery, without significantly affecting the proportion that recover (Table 4.1). It was hypothesised that a greater proportion of juveniles would be removed from a standard settlement substrata at higher concentrations of benzocaine and after longer exposure times and that fewer juveniles would recover from treatments of higher concentration and longer exposure. The proportion of juveniles removed from the substrata and those recovered from anaesthesia were analysed separately as the two were not independent.

Three concentrations of benzocaine (0.5, 1.0 and 2.0 $ml.L^{-1}$ seawater, chosen around the effective concentration used in the pilot experiment described above) and a seawater control were used with groups of juveniles exposed to each concentration for 5, 10 and 20 minutes. Three replicate substrata (200 x 200 mm PVC sheet) holding ~200 juveniles (~2.5 mm) were exposed to each combination of concentration and exposure time treatment, in individual baths. Exposure time treatments were staggered by 20 minutes to allow for the assessment of individuals between treatments. After the allocated exposure time the substrata was gently agitated in the bath. Juveniles that were removed from the substratum were collected, rinsed in seawater, counted and placed on similar substratum in a bath receiving non-recirculating seawater to recover. Recovery of juveniles was assessed after 18 hours. This recovery time was used as it equated with the minimum time required for juveniles to be handled and held in a hatchery prior to their release or transport, i.e. overnight. Juveniles were classified as recovered if they could maintain their hold on the substratum whilst it was gently agitated in seawater for

~10 seconds. The temperature of the treatment solutions and seawater in the recovery baths was kept at 19^{o}C (± 0.5^{o}C) throughout the experiment.

4.2.2.2. Transport of juveniles

The transport of juveniles from the hatchery to a release location followed a protocol using outcomes from the anaesthesia experiment described above and procedures to minimise the handling of juveniles and the time they were out of flowing seawater. Batches of juveniles that were to be released were anesthetised, rinsed in seawater and the total mass (grams) of the batch was measured by weighing ~500 g portions until the entire batch was weighed. After weighing each portion, juveniles were held in 1 mm mesh bags suspended in flowing seawater. Five samples (~100 individuals) from each batch were weighed to the nearest gram, counted and the maximum shell length (SL; mm) of 20 individuals in each of the five samples was measured. These measures provided an average number of juveniles per gram and a range and average SL for a batch. Once measured all individuals within a batch were divided into equal weights (to the nearest gram) and placed into deployment devices (see below, Section 4.2.2.3) in a tank receiving non-recirculating seawater delivered to each deployment device via a manifold for between 12 - 18 hours, to support recovery from anaesthesia.

Juveniles were transported to a field release location from the hatchery in deployment devices packed between layers of damp foam in 68 L plastic bins. At a release location, bins holding deployment devices were filled with seawater and swum to the reef by divers. Divers then handled individual deployment devices and distributed them evenly throughout the release location. Deployment devices were secured within the substratum by wedging them into interstitial spaces among the reef and under boulders. Their orientation was generally horizontal with PVC devices placed on the substratum with the side containing the openings facing down. Detailed maps of release locations were drawn including the positions of sites where deployment devices were placed to ensure they could be relocated.

4.2.2.3. Release from deployment devices

Two types of deployment devices (hereafter called devices) were designed to release juveniles. The first design consisted of a 300 mm x 120 mm diameter, 10 mm square mesh wire cylinder clamped shut at each end, containing small (<40 mm dia.) crustose coralline algae (CCA) coated rocks that had been sourced from within the local area to which juveniles were to be released (hereafter called CCA device) (Plate 4.3). A polyvinyl chloride (PVC) device consisted of a 300 mm length of rectangular cross section (125 mm x 65 mm) PVC tube with three 30 mm holes drilled into the upper surface and mesh panels (1.5 mm) secured over the ends (hereafter called PVC device) (Plate 4.4). Mesh panels were removed by the divers at the release site.

To ensure that the delivery of juveniles to release sites resulted in juveniles moving off devices two experiments were designed to investigate the decline in the proportion of juveniles on CCA and PVC devices that were released to two separate field locations consisting of similar habitat (Table 4.1, Experiments 5 and 6). The number of juveniles on devices was counted from replicate devices sampled immediately prior to release and from each release location after 1 and 2 days and between 7-14, 15-28 and 29-60 days. Requirements of releasing abalone to reefs within 5 kilometres of the source of parent stock, hatchery production limitations and logistics meant that different size juveniles were used in each release experiment.

It was hypothesised that the proportion of juveniles held in the CCA and PVC devices would decrease through time. It was assumed that the proportion of juveniles remaining on devices was a direct measure of movement of abalone off devices and into the surrounding habitat. Observations at each site and subsequent experiments investigating the dispersal of abalone into the surrounding habitat (Chapter 5, section 5.2.3) supported this assumption.

Experiment 5 - CCA devices

Twenty CCA devices (each holding ~600 juveniles; 2 mm SL) were deployed to twenty sites within a single release location (25 x 35 m) within 2 hours of transport from the hatchery. Each device was placed at the centre of a site and separated from other devices by at least 3 m. Three replicate devices were sampled at each time with the exception of that planned between 15-28 days after release as access to the location was not possible due to a combination of logistics and sea conditions. No sample was taken 2 days after release as access to the location was not possible due to sea conditions. The sampling of a device consisted of a diver removing it from the substratum and placing it in a plastic bag *in situ*. Devices were transported as per boulders in experiment 3. In the laboratory each device was soaked in 70% alcohol, the ends unclamped and contents removed, CCA coated rocks were brushed and visually inspected ensuring all juveniles were removed. The alcohol solution was sieved (1 mm) and the contents stored in 70% alcohol. Samples were sorted under a dissecting microscope and all juveniles counted. The proportion of juveniles remaining on devices at each time was calculated as the number on the device at each time divided by the mean number of juveniles on devices prior to release.

Experiment 6 - PVC devices

In experiment 6 the same procedure was followed as described for experiment 5 with the exception of 43 PVC devices (each holding 1 800 juveniles; 7.5 mm SL) being deployed to sites within one release location (1 025 m^2) within 2 hours of transport from the hatchery. Each device separated from others by ~6 m. Five replicate devices were sampled at each time with the exception of that planned 2 days of release where no sample was taken due to rough seas.

Data analysis

All of the experiments were orthogonal designs, with the exception of experiment 3 where the factor 'Sub-site' was nested in 'Shelter'. In all models with the exception of that in experiment 3, factors were analysed as fixed factors. Proportional data was transformed using the function arcsine-square root. Cochran's *C*-test was used to test for heterogeneity of variances (Underwood 1997) and data was transformed as described in the table caption for the respective analysis. Analysis of variance (ANOVA) was used to test for differences among treatments.

Plate 4.1 The deployment pump consisting of (from left to right); SCUBA tank with air inlet hose; 35 L drum fitted with on/off tap (red tap) at the start of the hose; lid, fitted with coupling for connection to air inlet and internal hose (attached air-stone not shown) and external hose fitted with pressure relief valve (42 Kpa). Inset: diver holding the end of the hose with diver controlled on/off tap.

Plate 4.2 Two of the five physical shelters, each covering 1 m^2 of substrata and consisting of a weighted skirt (chain filled light coloured material at base) and the body of the shelter (woven plastic cloth) fitted with an eyelet and attached buoy, positioned on the reef.

Plate 4.3 Three CCA devices (~300 mm long) in the laboratory containing recently added juvenile abalone, prior to being placed in a seawater tank to allow for their recovery prior to release.

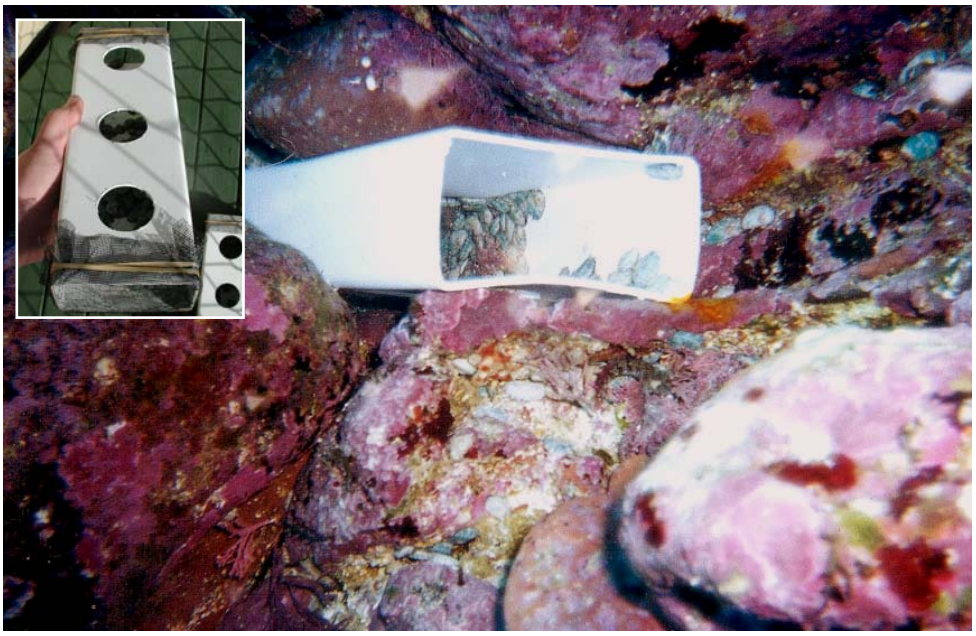


Plate 4.4 PVC device (300 mm long) *in situ*, holding juveniles (and juveniles in the surrounding habitat) about 18 hours after release. Note: the small boulder in the foreground was moved from the open end of the device to enable the photograph to be taken. Inset: PVC deployment device capped with 1.5 mm plastic mesh and containing juvenile abalone.

Table 4.1 Design of experiments investigating the supply of larvae through the deployment pump (Experiment 1), the effect of the deployment pump and physical shelters on the settlement of larvae (Experiments 2 and 3) and the handling and release of juveniles (Experiments 4 - 6). Treatments for each factor are shown in brackets and described in the text. Letters with experiment numbers are used to indicate independent batches of abalone, as applied within this chapter only.

Expt. No.	Factors [and treatments] in analyses	Size (mm)	Replicates	Response variable	Laboratory or field based
1a	Age (days) [7, 8] GABA [0, 1] Refrigeration [0, 24]	Larvae	4	No. larvae supplied (50 mL)	Lab.
2b, c	Time [1, 2] Drum [Full, Empty]	Larvae	3	Proportion settled after 24 hr	Lab.
3d	Shelter [0, 1] Sub-site [1, 2, 3, 4, 5]	Larvae	5	No. of larvae m^{-2}, after 24 hr	Field
4e	Benzocaine ml L^{-1} [0, 0.5, 1.0, 2.0] Exposure time (min) [5, 10, 20]	Juv (2)	3	Proportion removed & recovered	Lab.
5f	Days [0, 1, 2, 7-14, 29-60]	Juv (2)	3	Proportion of juveniles on devices	Field
6g	Days [0, 1, 7-14, 15-28, 29-60]	Juv (7.5)	5	Proportion of juveniles on devices	Field

4.3. RESULTS

4.3.1. Handling and release of larvae

4.3.1.1. Deployment pump

Experiment 1 - Supply of larvae from the deployment pump

Greater numbers of seven day old larvae were delivered from the drum of the deployment pump when they had not been exposed to refrigeration and this was consistent among treatments of GABA (Figure 4.1a and Table 4.2a). There was no significant difference in the number of larvae delivered from the drum among treatments of GABA (Table 4.2a). However, samples taken from the drum after the addition of GABA were more consistent. Variation among replicate samples taken from the drum after the addition of GABA ranged between 2-4% of the mean compared with 9-14% for those taken from the drum without the addition of GABA.

Results for eight day old larvae were not as consistent as those for seven day old larvae. A relatively low number of eight day old larvae were delivered from the drum where GABA was added and where larvae were not refrigerated (Figure 4.1b). This outcome resulted in a difference in the rank order of the mean number of larvae delivered among these treatments and resulted in a significant interaction term between the factors GABA and refrigeration in the analysis (Table 4.2b). In this experiment more eight day old larvae were delivered from the drum where they were not exposed to refrigeration within the treatment where they were not exposed to GABA (Figure 4.1b). This was similar to those patterns consistently observed for seven day old larvae.

The expected number of larvae delivered in 50 ml from the drum containing 35 L of water and 50 000 larvae was 71. This assumed all larvae were evenly distributed throughout the drum during the pumping process and all larvae were delivered from the deployment pump. The mean (± SE) number of eight day old larvae delivered from the start and end of the hose whilst the drum was full, half full and almost empty, among all treatment groups was 58 (± 3). However, within each combination of treatment groups that did not include refrigeration, the mean (± SE) number of larvae delivered per 50 ml was 74 (± 4) compared with 44 (± 3) for treatment combinations that included refrigeration. Similar results were observed for seven day old larvae where the mean (± SE) number of larvae delivered per 50 ml from all treatment groups combined was 57 (± 3), and among treatment combinations that did not include refrigeration the mean (± SE) was 58 (± 4) compared with 44 (± 4) among those treatment combinations that did include refrigeration.

Experiment 2 - Settlement of larvae delivered from the deployment pump

There was no significant difference in the proportion of larvae that settled after 24 hours on a standard settlement substrata after being delivered from the deployment pump when it was full and almost empty. This result was similar among both times where independent batches of larvae were used in the experiment (Table 4.3a and 4.3b). Between 41-48% and 59-61% of larvae settled after 24 hours at time one and two, respectively (Figure 4.2a and 4.2b). Variation among replicate samples was low among treatments for each time and ranged between 15-21% and 3-4% of the mean for time one and two, respectively.

4.3.1.2. Deployment shelters

Experiment 3 - Deployment shelters

There were significantly greater numbers of settled larvae.m^{-2} on the substratum under shelters 24 hours after the release of larvae (Figure 4.3 and Table 4.4). Between 30-173 larvae.m^{-2} were detected on substratum under shelters compared with between 0-22 larvae.m^{-2} on substratum, within the release location without shelter (Figure 4.3). Thus, more than 10 times as many settled larvae were detected on areas of reef where larvae were released under physical shelters compared with areas of reef without shelter. Less than 1% of released larvae were detected as having successfully settled, given that an estimated 20 000 larvae.m^{-2} were released to the location, including those to each shelter, and settlement was <200 larvae.m^{-2}.

4.3.2. Handling and release of juveniles

4.3.2.1. Anaesthesia

Experiment 4 - Removal and recovery of juveniles

The proportion of juveniles removed from a standard substrata differed significantly among concentration and exposure time treatments (Table 4.5a). Greater mean proportions of juveniles were removed using higher concentrations of benzocaine for longer periods of time, with the exception of that proportion removed at a concentration of 1.0 ml.L^{-1} for an exposure time of 10 minutes (Figure 4.4a). At concentrations ≥1.0 ml.L^{-1} more than 94% of juveniles were removed from the substrata, and after exposure at these concentrations for 20 minutes ≥98% of juveniles were removed. For the control and at a concentration of 0.5 ml.L^{-1}, <20% and <85% of juveniles, respectively, were removed from the substrata for all exposure times.

The proportion of juveniles that had recovered after 18 hours varied among concentration treatments but not among exposure times (Table 4.5b). Recovery of juveniles exposed to concentrations of 0.5 and 1.0 mg.L^{-1} ranged between 99-100% (Figure 4.4b). Recovery after 18 hours was reduced below or above these concentrations with between an average of 94-96%

of juveniles recovered within control groups and 91-97% recovered at a concentration of 2.0 mg.L^{-1} (Figure 4.4b).

4.3.2.2. Release from deployment devices

Experiment 5 - CCA devices

The proportion of juvenile abalone (2 mm SL) on CCA devices declined significantly through time (Table 4.6 and Figure 4.5). One day after release the mean proportion of juveniles on CCA devices declined to about 40% of the original 600 individuals released on each device. Numbers declined to <3% of those originally released after 7-14 days and <1% after 29-60 days. Variation among replicate samples was low and <25% of the mean, with the exception of that sample taken 7-14 days after release where it was 50%.

Experiment 6 - PVC devices

The proportion of juvenile abalone (7 mm SL) on PVC devices declined significantly through time following release (Table 4.7 and Figure 4.6). The mean proportion of juveniles remaining on PVC devices after 1 day was 60% of the original 1800 individuals. The decline in the proportion of juveniles on PVC devices reflected a steady decay with <1% of juveniles on PVC devices after 29-60 days of release (Figure 4.6).

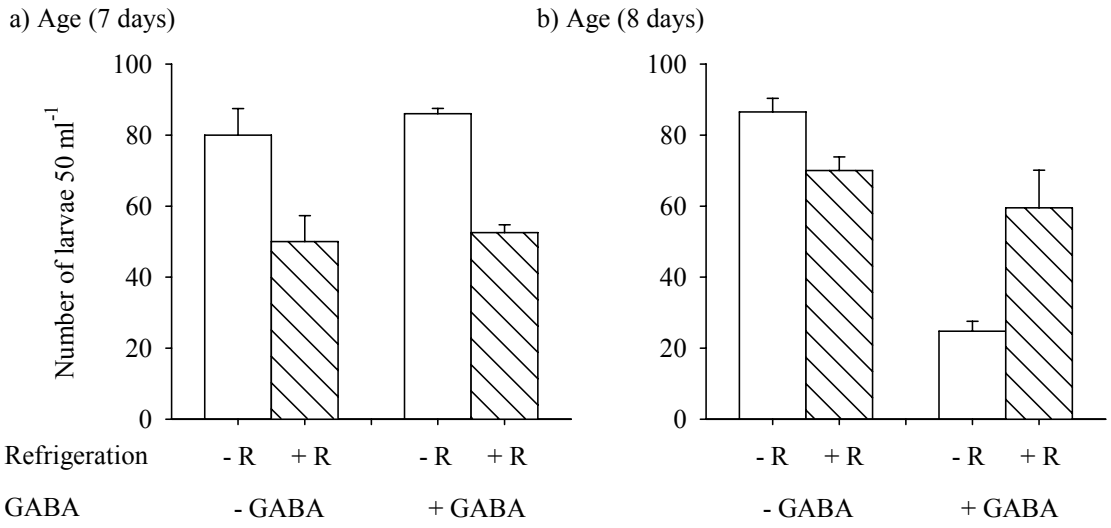


Figure 4.1 Experiment 1. Mean number of a) 7; and b) 8 day old larvae (+ SE) per 50 ml from the deployment pump with and without the addition of GABA and 24 hours of refrigeration (R) (n = 4).

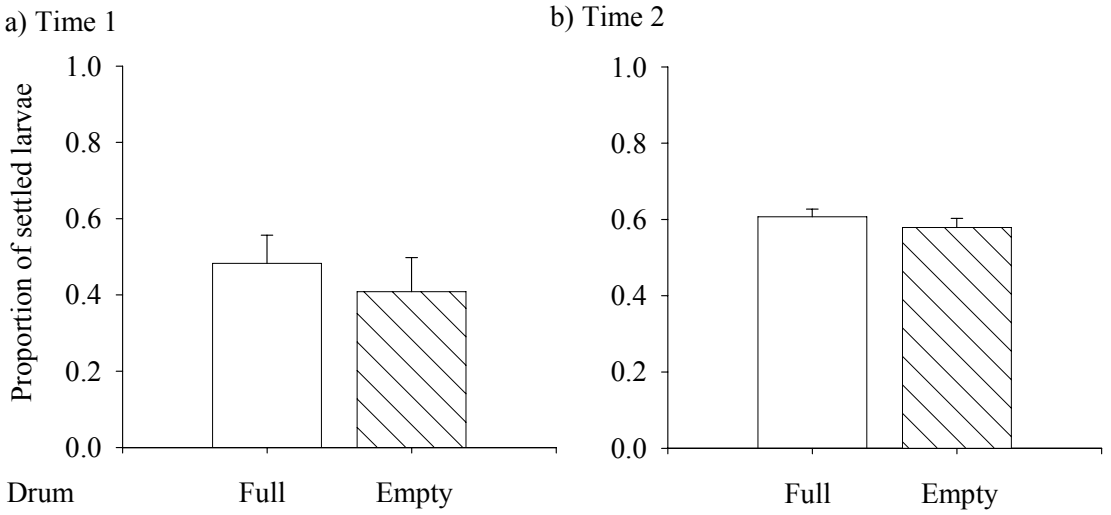


Figure 4.2 Experiment 2. Mean proportion of settled larvae (+ SE) on a standard settlement substrata after an exposure time of 24 hours, after being sampled from the deployment pump when it was full and almost empty (~5 L) from separate batches at two times a) Time 1; and b) Time 2 (n = 3).

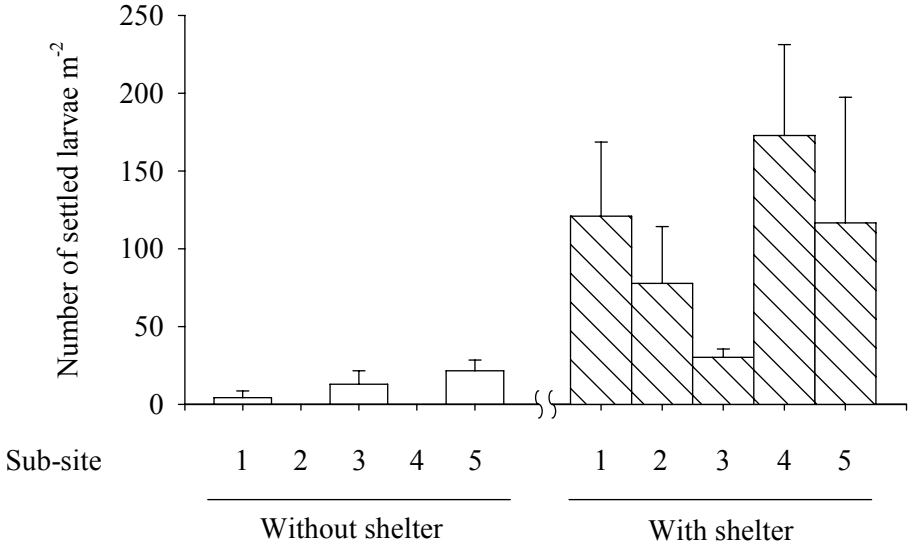
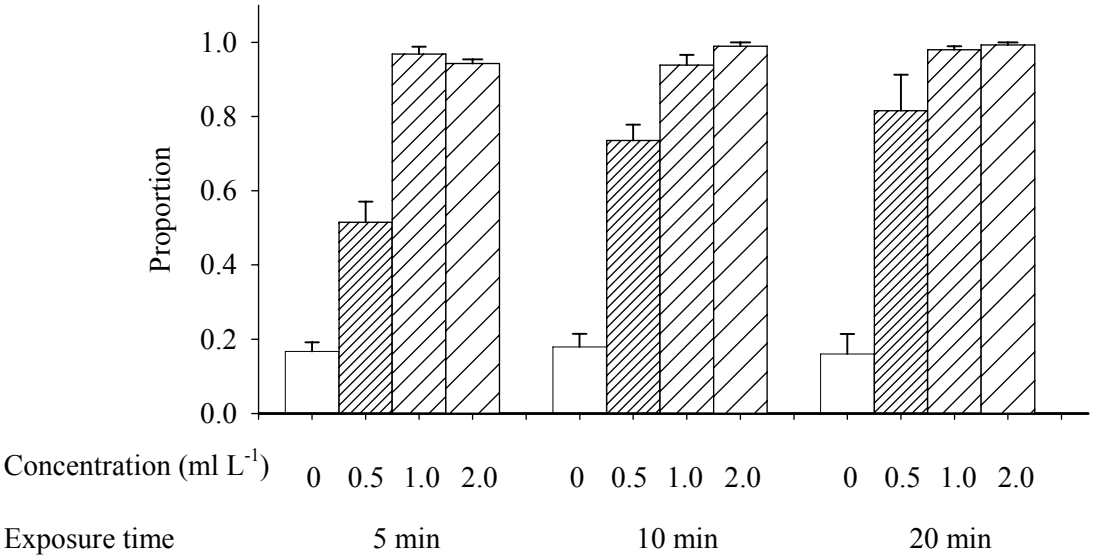


Figure 4.3 Experiment 3. Mean number of larvae.m^{-2} (+ SE) at 5 sub-sites without and with physical shelter, 24 hours after release (n = 5).

a) Removed from substrata



b) Recovered after 18 hours

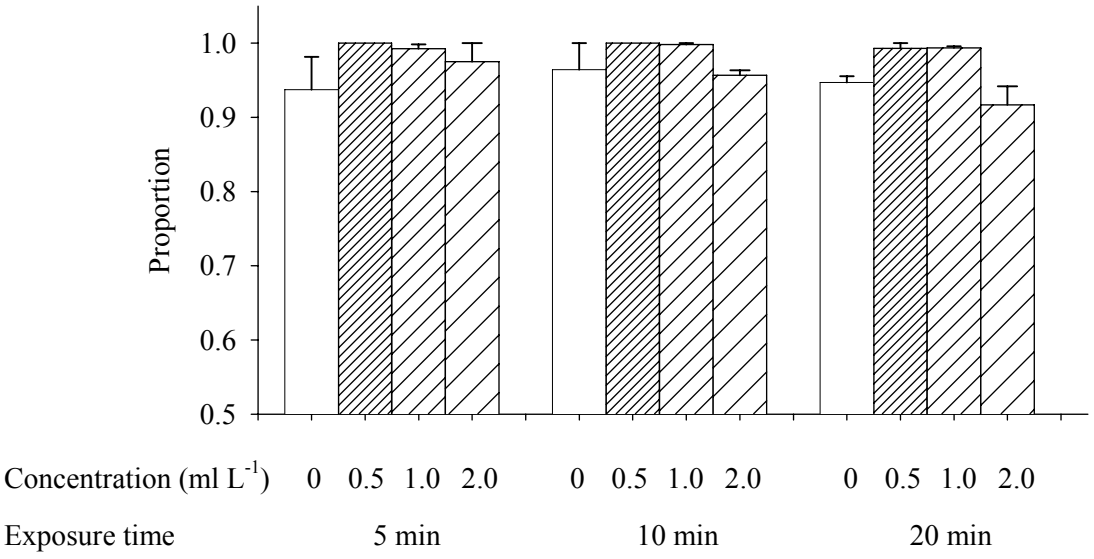


Figure 4.4 Experiment 4. Mean proportion of juvenile abalone (+ SE) a) removed from a standard substrata; and b) recovered from the anaesthesia treatments after 18 hours, using a control (seawater) and three concentrations of benzocaine over three exposure times (n = 3).

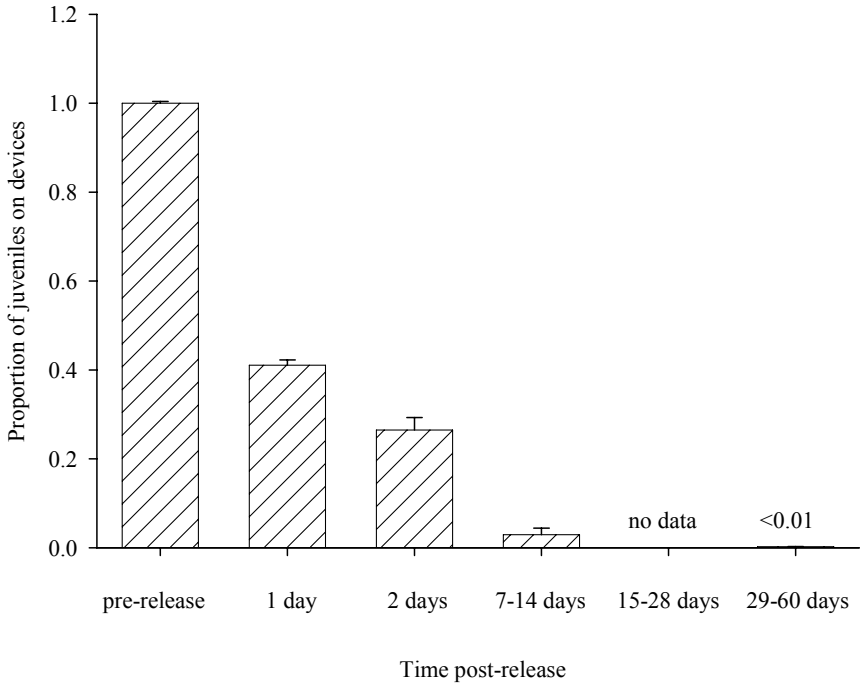


Figure 4.5 Experiment 5. Mean proportion of 2 mm juvenile abalone (+ SE) on CCA devices through time ($n = 3$). Note: A total of ~600 juveniles per device pre-release.

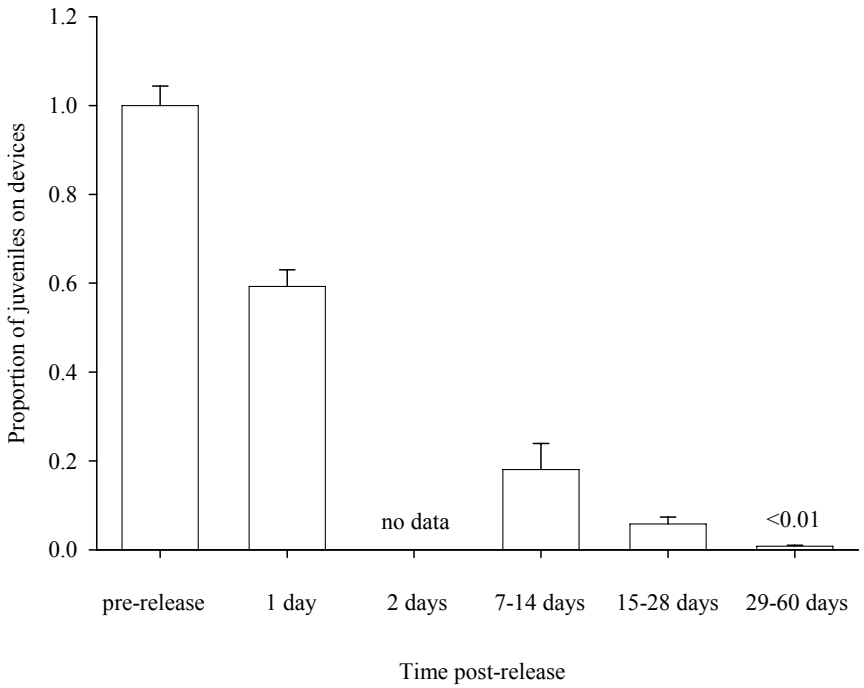


Figure 4.6 Experiment 6. Mean proportion of 7 mm juvenile abalone (+ SE) on PVC devices through time ($n = 5$). Note: A total of ~1800 juveniles per device pre-release.

Table 4.2a Experiment 1 – 7 day old larvae. Summary of analysis of variance in the number of 7 day old larvae delivered from the deployment drum in 50 ml samples. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.5429^{\dagger}$, ns; variances 4, df 3). †Square root (x+1) transformed.

	Source	df	MS	*F*		F vs
1	GABA, G	1	0.33	0.72	ns	4
2	Refrigeration, R	1	15.19	32.61	**	4
3	G x R	1	0.02	0.04	ns	4
4	Error	12	0.47			
5	Total	15				

Table 4.2b Experiment 1 – 8 day old larvae. Summary of analysis of variance in the number of 8 day old larvae delivered from the deployment drum in 50 ml samples. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.7341^{*\dagger}$; variances 4, df 3). †Square root (x+1) transformed.

	Source	df	MS	*F*		F vs
1	GABA, G	1	25.19	41.16	**	4
2	Refrigeration, R	1	2.91	4.76	*	4
3	G x R	1	12.73	20.79	**	4
4	Error	12	0.61			
5	Total	15				

Table 4.3a Experiment 2 – Time 1. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substrata after 24 hours at Time 1, after exposure to GABA (1×10^{-6} M) whilst the deployment drum was full. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.5712^{\dagger}$, ns; variances 2, df 2). †Arc-sine transformed.

	Source	df	MS	*F*	F vs
1	Drum, D	1	0.009	0.42 ns	2
2	Error	4	0.021		
3	Total	5			

Table 4.3b Experiment 2 – Time 2. Summary of analysis of variance in the proportion of larvae settled on a standard settlement substrata after 24 hours at Time 2, after exposure to GABA (1×10^{-6} M) whilst the deployment drum was full. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.6072^{\dagger}$, ns; variances 2, df 2). †Arc-sine transformed.

	Source	df	MS	*F*	F vs
1	Drum, D	1	0.001	0.78 ns	2
2	Error	4	0.002		
3	Total	5			

Table 4.4 Experiment 3. Summary of analysis of variance in the number of settled larvae.m^{-2} on natural substrata at 5 sub-sites with and without physical shelter, 24 hours after release. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.4049^{*\dagger}$, variances 10, df 4). †square root (x + 1) transformed.

	Source	df	MS	*F*	F vs
1	Shelter, Sh	1	527.171	24.44 **	2
2	Site (Sh)	8	21.569	1.32 ns	3
3	Error	40	16.293		
4	Total	49			

Table 4.5a Experiment 4 – Removal from substrata. Summary of analysis of variance in the proportion of juvenile abalone removed from a substrata after exposure to a control and 3 different concentrations of benzocaine for 3 exposure times. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.3184^{\dagger}$, ns; variances 12, df 2). †Arc-sine transformed.

	Source	df	MS	*F*		F vs
1	Concentration, C	3	2.065	171.88	**	4
2	Time, T	2	0.054	4.50	*	4
3	C x T	6	0.028	2.37	ns	4
4	Error	24	0.012			
5	Total	35				

Table 4.5b Experiment 4 – Recovery. Summary of analysis of variance in the proportion of juvenile abalone recovered after 18 hours after being removed from a substrata after exposure to a control and 3 different concentrations of benzocaine for 3 exposure times. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.3103^{\dagger}$, ns; variances 12, df 2). †Arc-sine transformed.

	Source	df	MS	*F*		F vs
1	Concentration, C	3	0.072	6.83	**	4
2	Time, T	2	0.021	2.03	ns	4
3	C x T	6	0.008	0.73	ns	4
4	Error	24	0.010			
5	Total	35				

Table 4.6 Experiment 5. Summary of analysis of variance in the proportion of 2 mm juvenile abalone on CCA devices at the time of release (day 0) and 1, 2, 10 and 42 days post-release. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.4123^{\dagger}$, ns; variances 5, df 2). †Arc-sine transformed.

	Source	df	MS	*F*	F vs
1	Time	4	0.986	373.46 **	2
2	Error	10	0.003		
3	Total	14			

Table 4.7 Experiment 6. Summary of analysis of variance in the proportion of 7.5 mm juvenile abalone on PVC devices at the time of release (day 0) and 1, 9, 23 and 57 days post-release. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.5252^{\dagger}$, ns; variances 5, df 4). †Arc-sine transformed.

	Source	df	MS	*F*	F vs
1	Time	4	1.473	134.09 **	2
2	Error	20	0.011		
3	Total	24			

4.4. DISCUSSION

The practical release of hatchery-reared abalone to natural reefs to enhance local populations requires a number of logistical processes, primarily those related to handling and preparation for release to be undertaken. These processes in addition to the method used to release abalone can have significant effects on their short-term (<60 days) survival (Leighton 1985, Tegner & Butler 1985, Schiel & Welden 1987, Tegner & Butler 1989, Schiel 1992, 1993, McCormick *et al.* 1994, Sweijd *et al.* 1998, Shepherd *et al.* 2000). The objective of the work presented in this chapter was to develop methods to deliver hatchery-reared *H. rubra* larvae and juveniles to natural reefs. The methods investigated demonstrated that large numbers of larvae, that were competent to settle could be released using the deployment pump. Further, the numbers of juvenile *H. rubra* on deployment devices at release sites declined through time such that <1% remained on devices after 30-60 days, with juveniles observed to move into the surrounding habitat from the time of release. The long-term survival of hatchery-reared and released *H. rubra* larvae and juveniles is assessed in Chapter 6.

4.4.1. Handling and release of larvae

Results from experiments investigating the effect of necessary handling and selected release treatments (recommended in Chapter 2 to support a release strategy) on the number of larvae delivered from the deployment pump demonstrated that the deployment pump provides an effective means of delivering larvae to a field location. However, some treatments such as refrigerated storage reduced the number of larvae delivered from the pump. The mean rates of larvae delivered from the pump indicated that about 80% are delivered to the reef. However, where the treatment combination included the addition of GABA and refrigerated storage, the percentage of larvae delivered from the drum could be as low as about 60%. The proportion of larvae not delivered from the pump is a consequence of their attachment on the internal surfaces of the drum and hose and those remaining in the residual contents of the drum (about 1 L) (personal observation). The likelihood of refrigerated storage affecting the short-term settlement response of larvae was demonstrated in laboratory experiments (Experiments 7 and 8, Chapter 2), with the proportion of larvae that settled within 60 seconds after 24 hours of refrigeration being more than double that of control groups. It is possible that a strong settlement response is affected by refrigerated storage resulting in the attachment of a larger proportion of larvae to the inside surfaces of the deployment pump. Finally, the proportion of larvae estimated to be delivered from the deployment pump is likely to be conservative, given that these results were based on samples obtained from adding a relatively small number of larvae to the drum (i.e. 50 000). It is likely that the proportion released would increase with a greater number of larvae

in the drum, assuming the surface area of the drum and hose on which the larvae attached is the limiting settlement surface where higher numbers of larvae are present.

Variation within and among replicate samples obtained from the pump, throughout the different stages of release was inconsistent (range 2-73% of the mean), and indicated the inadequacy of the deployment pump to deliver consistent numbers of larvae at low volumes. The inconsistent delivery of larvae at low volumes limits the application of the pump to support small scale field experiments where numbers of larvae <1 000s are required to be used. It is likely that the fine scale aggregation and 'tumbling behaviour' (Leighton 1972, Prince *et al.* 1987) of ready to settle larvae may reduce the likelihood of obtaining precise numbers at small volumes throughout the pumping process. Nevertheless, the small scale variation in the number of larvae delivered per 50 ml would have little affect on the regular distribution of larvae delivered at the spatial scale of a release location. At scales where the entire contents of the drum (35 L), or significant volumes of it are released, the small scale variation in the number of larvae delivered per 50 ml combined with active larval dispersal at fine spatial scales (Moss & Tong 1992a), would likely result in ostensibly undetectable patterns of fine scale variation in larval settlement.

The proportion of larvae delivered from the deployment pump that settled on a standard settlement substratum within 24 hours ranged between 40% and 60%. These proportions are similar, although marginally lower, to those reported for the same time to the same settlement substrata from controlled laboratory experiments (Chapter 2, Experiments 4-8 range: 53-80%) and support the assertion that the methods to handle and release larvae do not substantially affect their competency to settle, or their immediate survival. Moreover, it is likely that the proportion of larvae that actually settle at a field release location, given favourable conditions, would be significantly greater than indicated from these results as the magnitude of larval settlement to CCA covered substrata and other alga, expected at field locations, is greater than that to a standard settlement substrata (Experiments 1-3 in Chapter 2, Tong *et al.* 1987, Moss & Tong 1992a, Shepherd & Daume 1996, Roberts *et al.* 2004, Huggett *et al.* 2005).

The use of shelters at field locations significantly increased the number of larvae that settled after 24 hours. However, only a small proportion (<1 %) of those larvae released within sheltered sub-sites were detected on the substratum after 24 hours. This level of settlement is similar to those results reported by Preece *et al.* (1997) for *H. rubra*. They observed that between 0.03-2% of larvae were detected 19 days after release at three different densities and after they left shelters in place for 6 days. In contrast, results reported by Schiel (1992) after releasing *H. iris* at a similar density, using similar shelters for the same period of time were about an order of magnitude more (~10%) than those reported here for *H. rubra*. Despite significantly greater numbers of *H. rubra* being detected when using shelters and the possibility of improving this return through manipulation of release densities (Preece *et al.* 1997), the

logistic impediments and economic costs related to sheltering large areas of reef are restrictive. Nevertheless, the release of larvae at large spatial scales (>100s m^2) to areas of natural shelter within a release location and the efficacy of this larval release method needs assessment as a possible means of local population enhancement (Chapter 6).

4.4.2. Handling and release of juveniles

Methods tested to handle and release hatchery-reared juvenile *H. rubra* demonstrated that they could be effectively used to release large numbers of juveniles to natural reefs. The release of juvenile abalone from a hatchery requires a number of additional levels of logistic complexity compared with that for larvae. Juveniles require substantially greater area, volume and time to efficiently process them through procedures for their release including anaesthesia, quantitative assessment and distribution into manageable units to facilitate their recovery, storage and transport before finally being released to a field location. Integral to this process was the development and use of a simple physical structure (deployment device) that facilitated limited physical handling of juveniles and enabled known numbers to be successfully managed through to their release to natural reefs.

4.4.2.1. Anaesthesia

Anaesthesia of juvenile abalone using benzocaine has been documented in the literature for the primary use of removing them from a substratum (Hahn 1989a, Edwards *et al.* 2000). The proportion of juveniles removed using the concentration of 1 $ml.L^{-1}$ was similar to that at 2 $ml.L^{-1}$ (average difference <3%) and substantially greater than other treatments (Experiment 4). Further, the recovery of juveniles from the 1 $ml.L^{-1}$ concentration group exceeded 99% after 18 hours for all exposure times. The removal of *H. rubra* juveniles using benzocaine at 1$ml.L^{-1}$ was achieved within less than half of the time required to anaesthetise *H. rubra* described by Edwards *et al.* (2000) and one third of the time of that required to anaesthetise *H. iris* using the same concentration (Aquilina & Roberts 2000). However, varying methodologies between this study and those of Edwards *et al.* (2000) and Aquilina and Roberts (2000) pose difficulties in comparisons between these results. For example, this study described the use of anaesthesia to dislodge juveniles (2.5 mm) from the substrata after gentle agitation in seawater, and held at a temperature of 19^oC (± 0.5^oC), as opposed to that of Aquilina and Roberts (2000) where individuals (70-140 mm) detached from a vertical surface without assistance in seawater at temperatures of 15.5 - 15.8^oC and 21 - 22.5^oC, and Edwards *et al.* (2000) did not describe exposure times of less than 20 minutes.

Although the rate of anaesthesia of juveniles is concentration and exposure-time dependent and recovery can be affected by these factors, there are a number of additional factors that can affect

the anaesthetic and recovery response of individuals. For example, Aquilina and Roberts (2000) described a significantly faster anaesthetic response of *H. iris* to benzocaine at the same concentration in the warmer water treatment (21 - 22.5^{o}C). The results obtained in this chapter suggest that the use of benzocaine at a concentration of 1.0 ml.L^{-1} in seawater (19^{o}C) be used as the standard protocol for the removal of juvenile abalone for their handling and release. However, after extended application of this standard protocol, including in seawater temperatures ≥20^{o}C, it was modified to include the application of the lower concentration tested in experiment 4 (0.5 ml.L^{-1}), at these higher temperatures.

4.4.2.2. Transport of juveniles

The standard transport methodology of moving juveniles directly from the hatchery or holding facility to the release location in bins either separated by layers of damp material or in holding devices has been described by a number of authors (Leighton 1985, Schiel 1993, McCormick *et al.* 1994, Sweijd *et al.* 1998, Guzman del Proo *et al.* 2004, Dixon *et al.* 2006). This methodology was adopted for a standard protocol to transport juveniles from the hatchery to release locations where the time that juveniles were out of seawater was <2 hours. There was no observation of mortality associated with this method of transport with the exception of that noted below specifically for CCA devices (estimated to be 3-5%, section 4.4.2.3)

In addition to the standard transport protocol used during the release of juveniles described in this chapter a number of experiments in this thesis (see Chapters 5 and 6) required the extended transport (>2 and up to 10 hours) of juveniles from the hatchery. Where this was required juveniles were transported in the PVC deployment devices packed between layers of damp foam in a thermally insulated container containing ice bricks and lined with a plastic bag filled with medical oxygen and sealed (a method used to commercially export live abalone and as similarly described by Tegner & Butler 1985, Tong & Moss 1992, McCormick *et al.* 1994). Following extended transport devices were removed from containers and held in seawater tanks at a commercial live abalone processing company for a minimum of 10 hours and up to 48 hours if sea conditions restricted their release. There was one exception to this transport method, where juveniles were transported in 80 µm mesh bags and transferred to CCA devices after their recovery from extended transport (Chapter 6, Experiment 5). Tegner and Butler (1985), Schiel (1993) and McCormick *et al.* (1994) describe similar methods where extended transport was required to move juveniles from a hatchery to a release location. Tegner and Butler (1985) transported individuals (*H. rufescens*) on foam sheets and handled these individuals into mesh bags for placement in holding tanks for 36 hours before their release. Schiel (1993) described the addition of wet seaweed to transport devices holding *H. iris* and not the use of oxygen, and McCormick *et al.* (1994) described the cool storage and transport of two batches of juveniles

(*H. fulgens*) in mesh bags inside bags containing oxygen for 48 and 8 hours respectively, followed by their recovery in tanks of flowing seawater for 24 hours prior to release. Tegner and Butler (1985) did not describe any significant mortalities associated with their method of extended transport. However, McCormick *et al.* (1994) reported mortality rates of 41% for those individuals transported for 48 hours and Schiel (1993) reported mortality rates of up to 47% in devices where *H. iris* were transported at densities of 1 000 individuals per device for up to 7 hours, although improvements in reducing this mortality were described with reductions in density to 500 individuals per device. The densities of juveniles that were exposed to extended transport in this thesis were ≤1 000 individuals per device. It was often observed after extended transport that juveniles were not active and their response to touch was sluggish, similar to that reported by McCormick *et al.* (1994) for juveniles transported for 48 hours. However, it was unusual to observe any mortalities after periods of extended transport of those juveniles transported for release as described in this thesis. Further, after immersion in holding tanks (for between 10 and 48 hours) juveniles had recovered and were observed to be firmly attached to devices, which is in contrast to the low recovery after immersion for 24 hours of those juveniles transported for 48 hours reported by McCormick *et al.* (1994).

4.4.2.3. Release from deployment devices

The numbers of juveniles on devices at release sites decreased steadily through time and observations from experiments done in this chapter, and results from experiments investigating the dispersal of juveniles from devices described in Chapter 5, indicated that they move into the habitat immediately surrounding devices. Goodsell *et al.* (2006) described the rapid (>90%) movement of up to 200 *H. rubra* (10-12 mm SL) from cylindrical PVC devices in a laboratory experiment. Similarly McCormick *et al.* (1994) described the 'usual' movement of between 90-95% of 200 juveniles (25 mm SL) from a similar PVC device to that used in this study after 1-2 days, although they note that devices were left at release sites for up to 30 days for juveniles to move off them. Leighton (1985) described the relatively rapid movement of juvenile *H. fulgens* (10-25 mm SL) from a multilayered PVC device, with 10 and 2 individuals from a total of 29 remaining after 16 and 24 hours, respectively, and noted a study where time lapse photography recorded the movement of juvenile *H. rufescens* off these devices after 48 hours. The results reported by Leighton (1985), McCormick *et al.* (1994) and Goodsell *et al.* (2006) are in contrast to those reported for both CCA and PVC devices used in experiments in this chapter. Results from experiments reported here describe between 40-60% of juveniles remaining on devices after 1 day. Further, 26% of 600 juveniles (2.5 mm SL) remained on CCA devices after 2 days, although this proportion decreased to ~3% after 7-14 days, and on PVC devices 18% of 1800 juveniles (7.5 mm SL) remained after 7-14 days. It is possible that differences in the size and density of juveniles on devices as well as differences in structure of

devices affect the rate at which juveniles move from them. That juveniles used in these experiments were <10 mm SL may limit the generality of these results to the dispersal of larger juveniles from deployment devices. However, other factors a also likely to play a role in the differences in dispersal of juveniles in these experiments and from others using a deployment device. McCormick *et al.* (1994) held juveniles on mesh bags within devices and also oriented devices more vertically than done in this study. The PVC devices described by Leighton (1985) consisted of layers of thin corrugated PCV sheets. The juveniles remaining in PVC devices in this study were observed aggregated along the internal 90^{o} angles created by the rectangular shape of the PVC tube. It is possible that differences in physical structure of devices such as the internal angles and the three solid, smooth sides of PVC devices and the natural substrata of CCA devices used in experiments in this study, in addition to their horizontal orientation and securing them among boulders when released, provided a more stable environment than that of devices used by Leighton (1985) or McCormick *et al.* (1994) therefore reducing the time taken for juveniles to move off them.

Despite the potential benefits of the CCA device to provide a large surface area and increased physical protection from predators, whilst also providing a limited food source to small juveniles, there were several substantial logistic constraints to its use. The device required juveniles to be extensively handled after transport in mesh bags to be added to CCA devices. Further, by using CCA-coated rocks, sourced from reefs to which juveniles were being released, substantial effort was required to collect, handle and transport the large volumes of CCA-coated rocks required to construct the devices. Finally, although the logic of the design was to have the wire tube filled with rocks to stop their movement during transport, the settling of rocks and malleability of the wire mesh enabled rocks to move within the wire mesh and subsequently resulted in physical damage to juveniles during transport. Although the level of this damage was not quantified, direct mortality as a consequence of it was estimated to be 3-5% by the time they reached the release location.

Significant operational advantages of the PVC device included; 1) large numbers of anaesthetised juveniles could be added to it with minimal handling; 2) once added to the device juveniles did not have to be directly handled again; 3) large openings enabled large volumes of seawater to pass though it allowing recovery of anaesthetised juveniles as well as providing exit points; 4) greater numbers and larger sizes of juveniles could be held in it compared with the CCA device; 4) it was light weight and could be efficiently stacked in recovery tanks and during storage and transport; and 5) it was slightly malleable allowing it to be securely wedged among interstitial spaces and under boulders within a release location.

The release of juveniles in devices to a release location for experiments described in this thesis required <1 diving hour (*i.e*. two people for ~30 min. each). This effort compares favourably to

that reported by Schiel (1993) where he reported the release of 10 000 juveniles taking four diving hours, although this was where juveniles were removed from mesh bags by hand and placed on the reef. This comparison demonstrates that significant benefits in terms of time and financial savings can be made to a program where the release of juveniles using a device can provide comparable or improved results in terms of survival of released juveniles. Further, the placement and securing of devices within a release location as described in this chapter ensured they remained in place even after significant storm events, and where some devices were inadvertently left at the release location they were found during subsequent visits during long-term sampling and had not moved in >6 months (personal observation).

Regardless of the minor levels of mortality experienced by juveniles at or immediately after the placement of devices to natural reefs the numbers of juveniles on devices decreased steadily as a primary consequence of movement off them as demonstrated by the substantial numbers of juveniles observed in the surrounding habitat. These results demonstrate that these devices can be used to release large numbers of hatchery-reared juveniles, within relatively short periods of time, to natural reefs.

4.4.3. Conclusion and recommendations

The use of a deployment pump to release hatchery-reared larvae that are competent to settle provides the most reliable method to evenly disperse a large number of larvae over a broad area of reef. This method also provides the ability to direct the small scale distribution of larvae within close proximity to the substratum and amongst niche habitats where it is more likely that larvae will successfully settle, whilst reducing the loss of larvae to visual predators and possibly advection from the release area. Alternative methods of releasing larvae over large areas demonstrated limited practical application (Section 4.2.1). Despite the inability of the larval deployment pump to deliver 100% of larvae from the drum, the advantages of using this method suggest its successful use in releasing larvae to large areas of reef to assess their long-term survival, as presented in Chapter 6.

The standard protocol for the handling and release of larvae included the assessment of the total number of larvae and their concentration into a 1 L container (Chapter 2, section 2.2.5). Larvae were transported to a release location in the 1 L container, inside an insulated container containing an ice brick (section 4.2.1). At the location the 1 L container was placed in the drum of the deployment device that contained 34 L of seawater and allowed to acclimate to ambient seawater temperature (~20 mins). Once larvae were acclimated, GABA (1×10^{-6}M) was added to the seawater in the drum, thoroughly mixed and larvae were poured into the drum. The use of GABA for the release of larvae to the field was continued due to strong supporting literature describing its affect to increase the rate of settlement, despite limited evidence from results in

this chapter. The drum was sealed, pressurised and the tap on the drum, and end of the hose, opened. A diver directed the flow of larvae close to the substratum (<150 mm) from the end of the hose, whilst swimming to ensure an even coverage throughout the release location until the drum was emptied (~16 min.) (section 4.2.1.1).

Hatchery-reared juvenile abalone can be successfully handled, transported and released to areas of natural reef using methods, including the use of deployment devices, described in this chapter. The use of a deployment device reduces the physical handling of juveniles through the stages required to process them from a hatchery facility to a release location. Further, the use of devices to release large numbers of juveniles substantially reduces the time it would otherwise take to place them throughout a release location. However, the use of devices does result in small aggregations of juveniles at sites distributed throughout a release location. The effect of this heterogeneous distribution of juveniles on the precision and accuracy of sampling methods to assess long-term survival requires consideration (as addressed in Chapter 5), although it is unlikely that this initial distribution would influence assessment over long periods of time (years). The use of a PVC device to release juveniles, as described in this chapter, provides a simple, cost effective and efficient method to successfully release large numbers of juveniles to large areas of natural reef that other methods of release do not provide.

The standard protocol for the handling and release of juveniles included the anaesthesia of the batch of juveniles using benzocaine at 1.0 $ml.L^{-1}$ in seawater for 10 minutes (or 0.5 $ml.L^{-1}$ if ambient seawater was $\geq 20^{o}C$; section 4.2.2.1). Juveniles were rinsed, weighed and measured and equal quantities were divided amongst PVC devices that were capped with 1 mm mesh. Devices were placed in a tank of flowing seawater and seawater was directed into each device for 12-18 hours (section 4.2.2.2). After recovery from anaesthesia, devices were transported to a release location packed between layers of damp foam in 68 L plastic bins and swum to the reef by divers (section 4.2.2.2, or as described for extended-transport, section 4.4.2.2). Divers haphazardly distributed devices to sites throughout a release location, or as specifically described for each release. They were positioned horizontally with the access holes directed downwards, firmly secured within the substrata and the mesh capping removed (section 4.2.2.2). Devices were recovered from the reef during surveys to assess long-term survival to reduce the impact of releases on natural reefs.

5. DEVELOPMENT OF A MONITORING STRATEGY FOR RELEASED *HALIOTIS RUBRA* LARVAE AND JUVENILES

5.1. INTRODUCTION

One of the fundamental requirements of a stock enhancement program is the ability to measure the success of its objectives (Blankenship & Leber 1995, Munro & Bell 1997, Hilborn 1998, Howell 1998, Lorenzen 2005). An important objective of many of these studies is to accurately measure the survival rates of released individuals. Thus, the development of an effective monitoring strategy to quantify the number of released individuals surviving is critical.

Patterns of small-scale (<10 m) distribution and abundance of the different life history stages of abalone vary through time. Different life history stages exhibit different preferences for niche habitats, in addition to numerous other processes affecting patterns of distribution (Shepherd 1973, Connell 1985, Tegner & Butler 1985, 1989, Naylor & McShane 1997, Shepherd 1998, Dixon *et al.* 2006). For example, the larvae of several haliotid species show a preferential settlement response to crustose coralline algae and different growth forms of these species, as well as other specific alga (Shepherd & Daume 1996, Daume *et al.* 1999, Daume *et al.* 2000, Roberts *et al.* 2004, Huggett *et al.* 2005). Many of these habitats occur on exposed surfaces of solid substratum. As early juveniles develop and their diet habit changes from grazing on micro-algae to trapping and feeding on drift alga, they develop a more cryptic nature, inhabiting interstitial spaces under and between boulders, and in cracks and fissures in otherwise planar solid substratum (Shepherd 1973, Prince & Ford 1985, Shepherd & Turner 1985, McShane & Smith 1988, McShane 1991, Tarr 1995, Sasaki & Shepherd 2001, Dixon *et al.* 2006). Larger individuals are commonly found on more exposed areas of reef than juveniles, although adult *H. rubra* are generally more cryptic than a number of other species.

Methods to develop cost-effective surveys to quantify the abundance of subtidal, sedentary marine organisms are well established and widely accepted (Andrew & Mapstone 1987, Underwood 1997, Drummond & Connell 2005). The sample units allocated for surveying subtidal habitats can vary considerably but have traditionally consisted of areas defined within square (quadrat) or rectangular (transect) shapes, distributed randomly, haphazardly or systematically within a strictly defined habitat type (e.g. Barrens, *sensu* Underwood *et al.* 1991), or among defined strata within locations.

A number of sampling methods have been used to estimate the abundance of different life history stages of abalone. These methods have included a venturi-lift (McShane & Smith 1988), the collection and washing of small boulders (Prince & Ford 1985, Schiel 1992) and visual inspection of the substratum, with or without its disturbance e.g. turning over boulders, among a

range of sample units and defined strata (Sainsbury 1982, Shepherd 1985, Peck & Culley 1990, Andrew & Underwood 1992, McShane 1994, Schiel *et al.* 1995, Andrew *et al.* 1996, Gorfine *et al.* 1998, Cook & Sweijd 1999, Tarr *et al.* 2000, Chick *et al.* 2006, Mundy *et al.* 2006). The sampling method used, and its accuracy is, in part, influenced by the type of habitat being sampled. Each of the methods used has a range of limitations that restrict the ability to reliably and consistently estimate the abundance of abalone. For example, a venturi-lift requires the physical removal of settled larvae from the substratum using a combination of physical dislodgement and suction. The physical principles of this sampling method (i.e. the Venturi effect and Bernoulli's principle) dictate that its efficiency is reduced at shallower depths. In addition, the physical process of sampling and the delicate structure of settled larvae suggest that a proportion of them would be damaged, and unidentifiable after sampling. Given the limitations and restrictions in sampling methods it is difficult to maintain reliable estimates of the abundance of a population using similar methods where the majority of individuals progress through their life history (from settled larvae and early juveniles, to juveniles, early adults and adults), and as different niche habitats are utilised by these different developmental stages.

The development of a robust strategy to monitor the survival and persistence of released abalone is complicated by interactions between: 1) changes in the distribution of abalone among habitat types through their life history; and 2) differences in size as they grow. Moreover, comparing multiple methods through time is further complicated by the conflict between the needs for: 1) regular and representative estimates of the abundance of released individuals; 2) sampling so as not to significantly affect subsequent estimates (particularly long-term estimates ultimately used to determine the success of a release); and 3) resource limitations.

Furthermore, the release of juveniles using deployment devices (defined in Chapter 4) to sites that are evenly distributed within a release location, means that the initial distribution of juveniles within the location is not homogeneous (Plate 5.1). Gaining a greater understanding of the distance and time taken by juveniles to disperse from devices and into the habitat within a release location is critical in the development of an effective sampling strategy to estimate rates of survival through time (Saito 1984, Werner *et al.* 1995, de Waal *et al.* 2003). It is essential that a sampling strategy 1) account for the initial heterogeneous distribution of juveniles within a location if sampling is within short periods of time after release; 2) determine a distance to separate deployment devices within a location, so that the distribution of juveniles is relatively homogeneous throughout a release location over time (Plate 5.1) and hence estimate the time from release when, 3) long-term sampling strategies, that assume a homogeneous distribution of juveniles within a location, can be used to support accurate estimates of abundance and survival, as reported in Chapter 6.

Finally, the large-scale disturbance of habitat can significantly affect the abundance of abalone (Sainsbury 1982, Schiel 1993, Worthington *et al.* 1998, Tegner *et al.* 2001, this study). For example, significant storm events and rough seas can move boulders and/or increase sedimentation and destroy established habitat and thus result in the high mortality of individuals within it, either directly through physical damage or indirectly through the loss of habitat and/or food. Where these events occur the survival of individuals is likely to be less on the margins of the disturbed area. Developing a flexible monitoring strategy to continue to obtain representative estimates of survival beyond these events is critical for accurate assessments of survival within a release location.

The objective of this chapter was to develop a monitoring strategy to enable estimates of the abundance of abalone, of different sizes, released to natural reefs, to be made though time. This was critical to assess the long-term success of releasing abalone to natural reefs, as described in Chapter 6. A series of experiments were done to determine a) the most suitable method for monitoring successful settlement and early, post-settlement establishment of released larvae (1<60 days); b) determine the dispersal of juveniles from a point of release so that approaches to long-term monitoring could be established; and c) the most appropriate approach for measuring abundance of released *H. rubra* over periods of weeks to years.

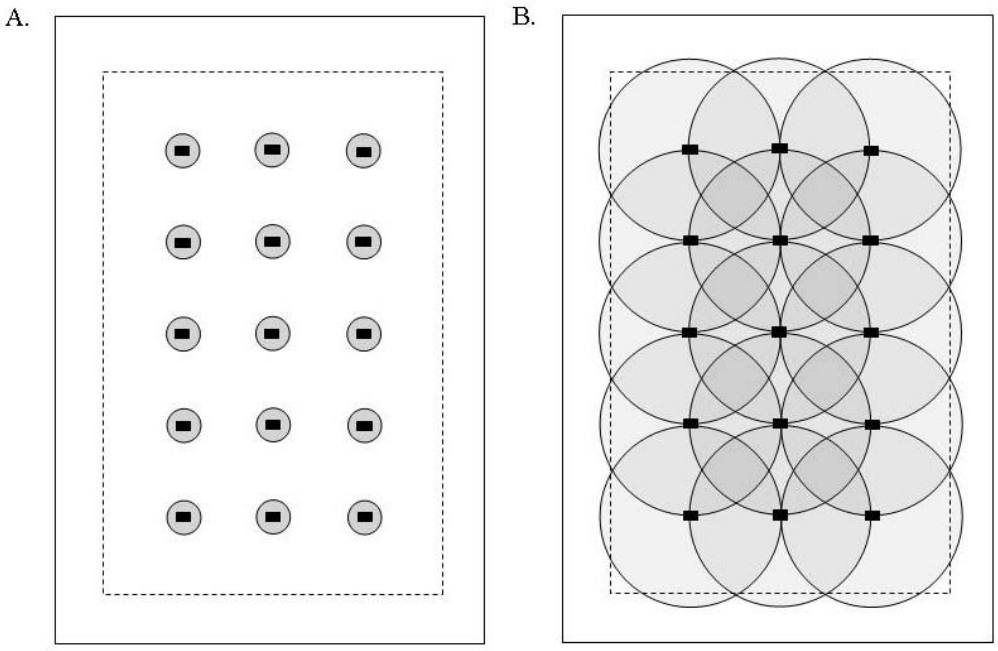


Plate 5.1 The idealised distribution of juveniles (grey shading) in uniform habitat shortly after release (e.g. hours; A) in deployment devices at 15 sites (small, solid black squares), regularly distributed within a release location (dashed box), allowing for their relatively even, theoretical distribution (circles) from each release site after a period of time (e.g. days; B), noting some migration from the survey location into surrounding area (solid box), not to scale.

5.2. MATERIALS AND METHODS

The approach and design of each experiment presented in this chapter was developed to determine an accurate monitoring strategy to quantify the settlement, early establishment and abundance of tagged and released abalone through time (Table 5.1). The reference to larvae, juveniles, and abalone throughout this chapter refers to larvae, juveniles or abalone that have been batch-tagged (see Chapter 3) and positively identified to be of hatchery origin, unless otherwise stated in the text. The methods used were a venturi-lift, the collection of small boulders, visual survey of the substrata *in situ* with no, low and high levels of substrata disturbance and stratified sampling within strata, based on the physical attributes of the habitat within a release location e.g. boulder field or solid habitat. Details of sampling techniques and standard protocols used are outlined below in Section 5.2.5 and 5.26, respectively.

5.2.1. Venturi-lift

Experiment 1

Experiment one was designed to investigate the effectiveness of the venturi-lift (Section 5.2.5 Sampling techniques) among different depths, to validate its use for sampling in subsequent larval settlement experiments. As the mechanism for generating lift was the expansion of compressed air at depth it was predicted that the venturi-lift would not generate equivalent suction at different depths and would sample more effectively at deeper depths. It was hypothesised that greater proportions of early juveniles would be sampled from a standard substrata at increasing depths using the venturi-lift.

A number of early juveniles (~2 mm; the maximum size estimated to be sampled using this method) were anaesthetised and 30 individuals were placed on each of 15 standard substrata, i.e. paving block (300 x 160 mm) and allowed to recover. Groups of five blocks were placed at depths of 2.5, 5 and 8 m (i.e. the likely range of depths over which samples would be taken from field locations using this method) and sampled immediately with the venturi-lift; $n = 5$ blocks. The number of juveniles on each block was counted prior to and after sampling. Numbers of juveniles in samples were compared with counts of juveniles on blocks before and after sampling to ensure no individuals were lost from the system.

5.2.2. Monitoring released larvae

Experiment 2

Experiment two was designed to compare two sampling methods to assess the short-term (~2 months) success of releasing larvae to a natural reef as determined by measures of density (number.m^{-2}) of settled larvae and early juveniles within the release location through time. The

two sampling methods were a venturi-lift and the collection of boulders (Section 5.2.5 Sampling techniques).

Given the limitations of the venturi-lift (reduced efficiency with decreasing depth and its potential to damage settled larvae), it was hypothesised that the density of settled larvae and early juveniles sampled using the collection of boulders would be greater than that using the venturi-lift, and that this would be consistent among sampling times. Further, that the density of settled larvae would decrease through time and this would be consistent among the sampling methods used.

Two million larvae were released in the location using the deployment pump (Chapter 4, Section 4.2.1 Handling and release of larvae). The location (~240 m^2) consisted of relatively loose boulders interspersed between areas of solid substratum within the Fringe habitat (Underwood *et al.* 1991, Andrew & Underwood 1992), that was not dominated by articulated coralline alga and other turfing alga, or containing large quantities of sediment among boulders (*sensu* boulder field). Venturi-lift samples and boulders were collected 1, 14 and 55 days after the release of developed larvae; $n = 5$ venturi-lift samples and boulders. As the efficiency of the venturi-lift differed with depth (Experiment 1) all samples taken using this method were restricted to a narrow depth range (4.5 m ± 0.5 m).

Experiment 3

Experiment three was designed to investigate the generality of results obtained in Experiment 2. It was hypothesised that the results of Experiment 2 would be consistent at three locations. Larvae (1.1 million) were released into three locations each ~375 m^2, consisting of a boulder field. Sampling was conducted as outlined for Experiment 2 with samples collected 2, 14 and 56 days after the release of larvae; $n = 5$ venturi-lift samples and boulders.

5.2.3. Monitoring released juveniles

Experiment 4

Experiment four was designed to investigate the dispersal of juveniles (~2 mm SL) from their point of release. This was done to inform the method of releasing juveniles in deployment devices and the likely distance to separate devices at release locations. It was predicted that the density of juveniles (number.m^{-2}) in the strata close to the point of release would be greater than that further away and that this pattern would be consistent among sites and through time (i.e. to 42 days). It was also predicted that the proportion of juveniles recovered in strata further from the point of release would increase as juveniles dispersed through time and this would be consistent among sites but that the total proportion of juveniles recovered would decline through time.

A CCA-rock deployment device (holding ~600 juveniles; ~2 mm SL) was placed at each of 20 sites within the release location (~150 m^2), with each site separated by at least 3 m. The release location consisted of a uniform area of solid habitat at a regular depth of about 6 m. At each time (1, 2, 10 and 42 days after release) three sites were randomly sampled. Sampling consisted of three replicate venturi-lift samples, haphazardly placed within each of two distance strata (0-0.3 m and 0.3-0.6 m). Each site was sampled only once throughout the experiment.

Experiment 5

Results from Experiment 4 provided evidence of the dispersal of 2 mm juveniles to at least 0.6 m within one day of release. This and observations of released juveniles (7 mm SL) moving up to 2 m within 1 day in a pilot experiment supported a larger scale investigation to define the spatial scale over which juveniles disperse and to determine the best spatial arrangement for juvenile releases to locations that would allow an even distribution within the location over time.

Experiment 5 was designed to investigate the dispersal of juveniles (11 mm SL) up to 10 m from their point of release through time. It was predicted that the proportion of juveniles recovered in distance strata encompassing the release point (0 - 2 m) would decrease through time and this would be consistent among sites. It was also predicted that the proportion of juveniles recovered among distance strata would become more homogeneous through time and this would be consistent among sites.

Eight PVC-deployment devices (holding a total of ~5 500 juveniles; 11 mm SL) were positioned within 0.5 m^2 of the centre point at each of three sites (~314 m^2; 10 m radius from release point). Locations were separated by ~150 m. Sampling consisted of visual surveys (Section 5.2.5 Sampling techniques) along four transects (10 x 0.5 m) that radiated from the centre of the release site (Gorfine *et al.* 1998). Each transect began from the centre of the site, with the first laid along a random bearing. Subsequent transects were offset from the first by 90^o. The total number of juveniles was counted in concentric distance strata, 0-2, 2-3, 3-5 and 5-10 m along each transect (see Plate 5.2), with the exception of the distance strata 0-2 m at 1 day post-release, where the numbers of juveniles were high (>200) and estimated to the nearest 50. The percent of area sampled within each distance strata was 8, 10 , 5 and 2, respectively. The small difference in representative area sampled among the distance strata was assumed to be negligible. This assumption was supported by the consistent detection of abalone within strata among sites through time. Further, to reduce variation in the number of abalone surveyed among sites and through time (Gorfine *et al.* 1998, Mundy *et al.* 2006) one diver completed all surveys. Deployment devices were left undisturbed for the duration of the experiment. Sites were sampled 1, 4 and 8 days post-release; n = 4 transects.

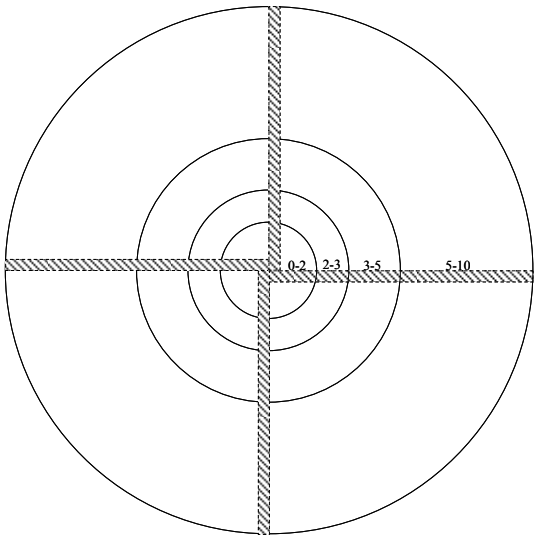


Plate 5.2 Sampling design within a release location where the dispersal of juvenile abalone was investigated within concentric distance strata 0-2, 2-3, 3-5 and 5-10 m from the central release site, as described in Experiment 5. Transect survey areas (10 x 0.5 m) are presented as hashed rectangles radiating from the central release site, offset at a 90^{o} compass bearing, not to scale.

5.2.4. Long-term sampling

Results from Experiments 4 and 5 identified the rapid dispersal of juveniles into the surrounding habitat from their point of releases, such that the release of juveniles to sites separated by small spatial scales (~6 m) within a location would provide substantial overlap in their distribution after a relatively short period of time (<1 month). This relatively even distribution within the location would then enable efficient and effective monitoring of their abundance over long periods of time.

Experiments done to estimate the long-term (>2 month) survival of abalone within release locations were designed from experience quantifying the abundance of natural abalone populations and knowledge gained from the literature. Comparisons of the precision and relative accuracy of different sampling methods were done within replicated fixed area quadrats. The outcome of these comparisons was used in the development of a long-term monitoring strategy for releases of abalone in Chapter 6.

Experiment 6

Experiment six was designed to compare the precision and relative accuracy of the sampling methods, visual surveys (VQ), boulder sampling *in situ* (B*is*) and boulder sampling with disturbance (BQ; Section 5.2.5 Sampling techniques) in a boulder field. It was hypothesised that more consistent and greater number of juveniles would be sampled using methods employing more thorough search techniques (i.e. BQ>B*is*>VQ). Precision was measured as the standard

error as a percentage of the mean. The mean precision of each method was generated from twenty samples, taken with replacement, from the set of 15 replicates using each method.

Thirty-two deployment devices (each holding ~780 juveniles; 14 mm SL) were evenly distributed within a location consisting of a boulder field (~240 m^2). Sampling, using each of the three methods was done 93 days after the release of juveniles using quadrats (600 x 600 mm; for each method) haphazardly cast within the location; n = 15 quadrats.

Experiment 7

Abalone become less cryptic as they become larger and after longer periods of time the accuracy of visual survey methods, that do not require habitat disturbance, should increase. However, factors confounding the accuracy of these visual surveys include the size of individuals and habitat type, as smaller individuals and more complex habitat would potentially reduce the likelihood of detection. Experiment seven was designed to investigate the generality of results obtained using visual surveys (VQ) and boulder sampling with disturbance (BQ) in Experiment 6, with larger juveniles (40 mm SL). It was hypothesised that there would be no significant difference in the number of large juveniles surveyed three months post-release using visual surveys and boulder sampling with disturbance, and this would be consistent among locations.

At each of four locations (~250 m^2) about 700 juveniles (40 mm SL) were haphazardly released by divers hand placing individuals on substrata within a boulder field, ensuring each individual was attached. Sampling, using each of the two methods, was done ~122 days after the release of juveniles using quadrats (1 x 1 m) haphazardly and independently cast for each method, within the location; n = 5 quadrats.

Experiment 8

Experiment 8 was designed to investigate the accuracy of using disturbed boulder sampling within two strata of a release location to determine the abundance of released abalone after boulder field habitat had undergone substantial natural disturbance (within about 4 months, inferred from a lack of substantial algal growth on the surface of disturbed boulders). The two strata consisted of disturbed boulder field and solid habitat (Section 5.2.5 Sampling techniques, Stratified sampling). It was predicted that the density of abalone sampled within solid habitat would be greater than that in disturbed boulder field habitat, and that this would be consistent between locations within areas.

At each location (~100 m^2) within each area about 1 000 and 9 000 juveniles (~15 mm SL) were released using deployment devices, respectively. Sampling was done ~770 days post-release using quadrats (1 x 1 m and 600 x 600 mm at locations within areas 1 and 2, respectively) haphazardly cast within each stratum at each location; n = 5 quadrats.

Sampling techniques

Venturi-lift

A venturi-lift can be used to estimate the abundance of settled larvae and early juveniles (McShane & Smith 1988, Wahle & Steneck 1991). The venturi-lift used in these studies was adapted from that described by McShane and Smith (1988) and consisted of; (1) a 1500 mm length of 40 mm diameter PVC tube; (2) a high pressure hose fitting with a ball-valve fitted near the end of the tube, to control the introduction of air (from a SCUBA tank) and to generate air-lift suction; (3) a length of 45 mm diameter hose with a brush attachment; and (5) a mesh (180 μm) bag fitted to the end of the PVC tube, to retain the material lifted after brushing the substratum (Plate 5.3).

Samples taken using the venturi-lift consisted of opening the air inlet valve on the venturi-lift and brushing all substrata that could be reached within a sample quadrat (250 x 250 mm) haphazardly placed within a location (with the exception of samples taken in Experiment 1 from a standard substrata). Brushing of the area within each quadrat was for ~30 seconds (in small circular movements), allowing the area within the quadrat to be covered with 2 passes of the brush. Immediately following sampling the air flow was stopped and the mesh sample bag was inverted, removed and sealed inside a labelled jar *in situ*.

The contents of each sample bag was thoroughly rinsed and sieved (180 μm) in the laboratory and stored prior to sorting (Section 5.2.6.1 Laboratory protocol). A quadrat was not sampled if the habitat lying within it contained more than a sparse, single layer of sediment, or if it contained large foliose or geniculate algae. Where a quadrat was located in poor habitat it was moved to the nearest suitable habitat.

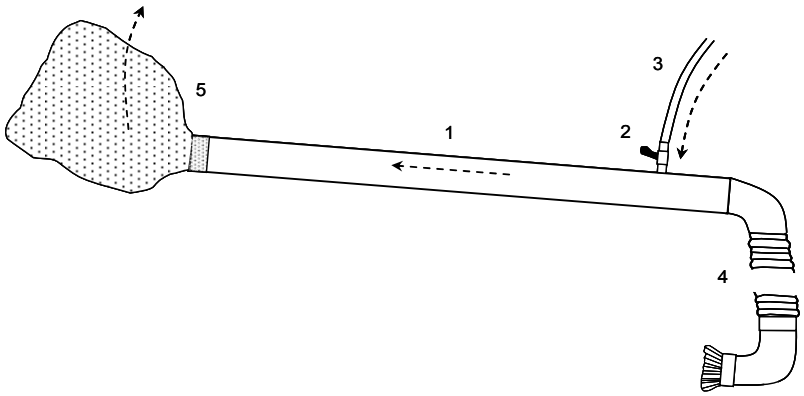


Plate 5.3 Venturi-lift consisting of: 1) 1 500 mm length of 40 mm dia. PVC tube; 2) ball-valve; 3) high pressure hose to SCUBA tank; 4) length of 45 mm dia. plastic flexible hose with brush attachment; and 5) 180 μm mesh sample bag with Velcro® strap. Arrows indicate direction of air-flow. Not to scale. Modified from McShane and Smith (1988).

Visual surveys

Visual surveys are a long established method for measuring the abundance of wild abalone populations, including *H. rubra*, without disturbing the habitat or the abalone (Shepherd 1985, Andrew & Underwood 1992, McShane 1994, Andrew *et al.* 1996, Hart *et al.* 1997, Worthington *et al.* 1999). Although limitations of visual methods to sample juvenile abalone have been acknowledged they do provide a means of obtaining repeatable measures of the abundance of juvenile abalone, particularly those greater than ~20 mm in length (Prince & Ford 1985, McShane & Smith 1988, Shepherd 1998).

Visual census of the sample area within a release location, either along a transect (Experiment 5) or within a quadrat (Experiments 6 and 7) consisted of the thorough visual inspection of the substratum by an experienced diver trained in the observation of small abalone. During this inspection no substratum was disturbed and all abalone were counted. Where any part of an abalone fell within the plane above or below the defined survey area it was counted.

Boulder sampling

A variety of methods of sampling boulders have been used to estimate the abundance of wild abalone from newly settled larvae through to large juveniles. These include the collection and washing of boulders in alcohol, the visual inspection of individual boulders *in situ* by lifting and replacing them, and the sampling of fixed areas within boulder habitats through the deconstruction of the boulder field within the fixed area (Sainsbury 1982, Prince & Ford 1985, McShane & Smith 1988, Prince *et al.* 1988b, Schiel 1993).

The boulders sampled were representative of those within a boulder field and had a maximum diameter of ~300 mm. Where boulders were sampled from a location without the use of a quadrat to define a sample area, such as the boulder collection method and the general application of the boulder *in situ* method (see below), the mean density of boulders was measured by casting quadrats (1 x 1 m or 600 x 600 mm) within the release location; n = 5-15 quadrats (as described for each experiment). The density of abalone within a location was calculated for each replicate as the number of abalone on the boulder multiplied by the mean density of boulders within each location.

Sampling in a fixed area (quadrat) within a boulder field required relocation of the quadrat when it included an area of habitat that could not be sampled, unless otherwise stated for specific experiments (see below, Stratified sampling). Where this was necessary the quadrat was moved to the nearest available area to allow a complete sample.

Samples taken using these boulder sampling methods were assumed to be representative of the entire release location, (with the exception of samples taken within disturbed boulder and solid habitat at a release location as described below, Stratified sampling). This assumption was

supported by choosing locations that minimised the area that was not boulder field habitat and observations within areas of solid habitat e.g. among large boulders, generally unable to be sampled using this method, that supported individual and small aggregations of abalone within locations, similar to those observed within the boulder field.

Boulder collection

Where boulder collections were done single boulders were collected from the top layer of boulders only (where there was more than one layer) and placed in a plastic bag and sealed *in situ*. Bags containing boulders were placed on a layer of sponge in a plastic bin. Bins were swum to a support vessel and samples returned to the laboratory for processing within about four hours. Boulders were removed from the plastic bag, soaked and rinsed in 70% alcohol for between 10-15 mins and the surface gently brushed before being discarded. Each plastic bag, associated with each sample, was rinsed using 70% alcohol and discarded. The sample (particulate matter and 70% alcohol) was sieved (180 μm) and all particulate matter stored in clean 70% alcohol prior to being sorted (Section 5.2.6.1 Laboratory protocol).

Boulder sampling in situ

The use of sampling boulders *in situ* was done where juveniles were able to be visibly assessed (>~7 mm). This method involved the visual inspection of a haphazardly sampled boulder, from within the top layer of boulders only (where there was more than one layer) or from the top layer of boulders within a fixed area (see Experiment 6). Boulders were picked up, the abalone on them were counted, and the boulders were then replaced in their original position. Where epiphytic or other growth obscured the spire of the abalone shell and hence the identifying tag, it was removed to enable identification of the abalone, otherwise abalone were not removed.

Boulder sampling with disturbance

The sampling of boulder habitat with disturbance consisted of all boulders within the sample replicate being systematically picked up, inspected and temporarily removed from the immediate sample area until sampling was complete. Abalone on all of the rocks within the defined area were counted. Boulders were then replaced within the immediate sample area once sampling was complete.

Stratified sampling

A number of locations within this study were exposed to large-scale disturbances caused by rough seas. To obtain comparable estimates of the minimum number of juveniles surviving within a location through time, after the occurrence of one or more of these episodes, a stratified sampling approach was undertaken. Two strata were defined, stratum one consisted of boulder field habitat as previously defined. Stratum two, consisted of habitat that could not be physically

disturbed, i.e. boulders cemented together, immovable boulders and patches of solid bedrock with cracks and fissures (*sensu* solid habitat). Sampling within both strata was done using the method described for boulder sampling with disturbance (see above). However, in stratum two this method was modified so that a quadrat consisted of between 25 - 50% of solid habitat, with the remaining area within the quadrat sampled.

Samples taken within stratum two were assumed to be representative of that area defined as solid habitat within the release location. The proportion of abalone surviving was calculated as the quotient of the mean number.m^{-2} of abalone within sample replicates multiplied by the area of solid habitat within the release location (m^2; dividend) and the original number of abalone released (divisor). This area of solid habitat was calculated by multiplying the total area of the release location by the proportion of quadrats required to be randomly cast to obtain the required number of sample replicates that contained >25% solid habitat within each release location.

5.2.5. General protocols

5.2.5.1. Laboratory protocol

Samples taken from the field were stored in 70% alcohol. Where samples were taken from locations where larvae were released they were stored in the dark (to prevent the decay of the calcein labelled larval shell from UV light). Samples containing abalone estimated to be < ~7 mm were sorted using a dissecting microscope. The sample (or proportions of it, until the entire sample was sorted) was washed into a glass petri dish and the dish positioned under the microscope on a sheet displaying a 10 mm grid. The sample was thoroughly inspected and all abalone (or sub-samples, if samples consisted of large numbers of individuals) from samples taken in the field were positively identified as being tagged through identification of the calcein tag in the larval shell or the green-blue juvenile shell in the spire (described in Chapter 3).

Elutriation of samples

Samples collected using either the venturi-lift or the collection of boulders contained up to ~200 g of sediment and other material. The processing of these samples could take between 6 and 10 hours, using the methods described above. To reduce this time, a method of elutriation was tested using samples containing sediment, spiked with a known numbers of early juveniles (newly settled; 0.5 - 1 mm SL) and larger juvenile (1 - 2 mm) abalone.

Three treatments were used. The first had a known high number (~100) of early juveniles. The second, a known, low (~ 20) number of early juveniles and the third a known number (~50) of larger juveniles. Three replicate samples of each treatment were made up and sorted. Samples consisted of 15 g of sand and finer sediment in addition to the known number of abalone. The

treatment type and exact number of abalone in each replicate was unknown to the sorter. Each replicate was elutriated prior to sorting. This process involved the sample to be rinsed into a 2 L glass container that was suspended over a 180 μm sieve. The container was then filled with water from a 10 mm diameter hose at a flow rate of ~5 $L.min.^{-1}$. The flow of water was used to mix the sample, whilst overflowing the beaker. After ~1 minute flow was stopped and the contents of the sieve were rinsed into a sorting tray and the number of abalone counted using methods described above. The mean (± SE) proportion of small abalone from treatment 1 was 0.96 (± 0.02), treatment 2 was 0.83 (± 0.02) and treatment 3 was 0.99 (± 0.01).

Elutriation, prior to the standard sorting of samples, reduced sampling time by between 40 and 60% (to 2.5 – 3.5 hr per sample) whilst not substantially reducing the numbers of abalone detected. Elutriation of samples that contained large quantities of sediment (as determined by the sorter) was adopted as part of the standard laboratory sorting protocol.

5.2.5.2. Calculations of density and proportion survival

Where samples were taken from within a fixed sample area (quadrat) the mean (SE) $number.m^{-2}$ within a location was calculated from the product of the $number.m^{-2}$ from each replicate, the proportion of the release location sampled and the total area of the release location. Where samples were taken via the collection of boulders or the inspection of individual boulders *in situ* the mean (SE) $number.m^{-2}$ of abalone within a location was calculated from the product of the number of abalone on each boulder (n) and the mean number of $boulders.m^{-2}$ within the sample area. The mean number of $boulders.m^{-2}$ at each location was generated from counts of the number of boulders in independently cast quadrats.

The proportion of abalone surviving was calculated as the quotient of the mean $number.m^{-2}$ of abalone multiplied by the total release area (m^2) (dividend) and the original number of abalone released (divisor) (unless otherwise stated in the experimental methods).

5.2.5.3. Data analysis

Analysis of variance (ANOVA) was used to test for differences among treatments. All analyses consisted of orthogonal designs. In all models factors were analysed as fixed factors, with the exception of the factor Location in experiments 3 and 7, where it was analysed as a random factor. Cochran's *C*-test was used to test for homogeneity of variances and data were transformed as described in the text. Where data were heterogeneous after transformation ANOVA was still performed on transformed data, as the method is robust to heterogeneity of data. Results were conservatively interpreted with $p<0.01$ (Underwood 1997).

Table 5.1 Outline of field experiments to investigate sampling methods and juvenile dispersal. Treatments for each factor are shown in brackets and described in the text. Common letters with experiment numbers are used to indicate experiments completed with the same batch of abalone, as described within this chapter only. Sampling methods, described in the text include the venturi-lift (V), visual surveys along transects (VT), visual surveys within quadrats (VQ), boulder collection (BC), boulder sampling *in situ* (B*is*), disturbed boulder sampling (BQ) and disturbed boulder samples in boulder field (B) and solid (S) habitat.

Expt. No.	Factors [and treatments] in analyses	Size at release (mm)	Estimated size at sampling (mm)	Days sampled after release	Sample method	Habitat type	Locations	Replicates	Strata sampled as distance from release point (m)
1a	Depth (m) [2.5, 5, 8]	2	2	NA	V	standardised substrata	1	5	NA
2b	Method [V & BC] Days [1, 14, 55]	Larvae (0.3)	0.3-2	1-55	V & BC	boulder field & solid	1	5	NA
3c	Method [V & BC] Days [2, 14, 56] Location [1, 2, 3]	Larvae (0.3)	0.3-2	2-56	V & BC	boulder field & solid	3	5	NA
4d	Days [1, 2, 10, 42] Site [1, 2, 3] Distance (m) [0.3, 0.6]	2	2-5	1-42	V	solid	1	3	0-0.3, 0.3-0.6
5e	Days [1, 4, 8] Site [1, 2, 3] Distance (m) [2, 3, 5, 10]	11	11	1-8	VT	solid	3	4	0-2, 2-3, 3-5, 5-10
6f	Method [VQ, B*is* , BQ]	14	35	93	VQ, B*is* & BQ	boulder field & solid	1	15	NA
7g	Method [VQ, BQ] Location [1, 2, 3, 4]	40	45	122	VQ & BQ	boulder field & solid	4	5	NA
8h	Area [1, 2] Location [1, 2] Strata [1, 2]	15	70	~770	B & S	boulder field & solid	2	5	NA

5.3. RESULTS

5.3.1. Venturi-lift

Experiment 1

Depth significantly affected the number of early juveniles sampled from standard substrata using the venturi-lift (Figure 5.1, Table 5.2). An average of 92% of juveniles were removed from the substrata at 8.5 m. Samples taken at 2.5 and 5 m recovered 68% and 84% of juveniles, respectively, i.e. 74% and 91% of those at 8 m. (Figure 5.1). Variation among replicate samples was low among treatments, at less than 8% of the mean.

5.3.2. Monitoring released larvae

Experiment 2

Very high densities of settled larvae were detected on the substratum immediately after release (>600 m^{-2}; Figure 5.2). Densities of settled larvae were consistently greater in the boulder collection (BC) treatment at each time post-release. However, the magnitude of differences in the mean number of settled larvae.m^{-2} differed between the two sampling methods among sampling times, resulting in a significant interaction between the factors Method and Time (Table 5.3). Despite this, the density of settled larvae decreased through time and this was consistent among samples collected using the venturi-lift (V) and the collection of boulders (Figure 5.2). Venturi-lift samples recovered 4 - 26% of those taken using boulder collections 1 and 14 days after the release of larvae and none were detected at 55 days post-release. Variation among replicate samples was generally low for both treatments at between 3 - 31% of the mean.

Experiment 3

High densities of settled larvae were detected on the substratum early after release at each location (>80 m^{-2}; Figure 5.3). Analyses indicated that there was an inconsistent number of settled larvae.m^{-2} sampled by each method through time (Table 5.4). This was likely due to low and inconsistent densities detected between methods 56 days post-release. Within 14 days post-release the densities of settled larvae were greater in samples using boulder collections and consistently decreased through time among locations (Figure 5.3). Venturi-lift samples recovered 6 - 32% of boulder collections 2 and 14 days post-release. Variation among replicates was higher for samples taken using the venturi-lift (41 - 100 % of the mean) than the collection of boulders (32 - 47%) 2 and 14 days post-release, among locations.

5.3.3. Monitoring released juveniles

Experiment 4

Mean densities of juveniles were generally higher close to the point of release (0.3 m) within the first 10 days of sampling and this was consistent among the independent sites sampled; with the exception of Site 4 after two days (Figure 5.4). Densities of juveniles were in excess of 30 m^{-2} and 10 m^{-2} in the inner (0 - 0.3 m) and outer (0.3 - 0.6 m) strata, respectively, within the first 10 days, with the exception of Site 8 after 10 days. Low densities (<11 abalone.m^{-2}) of juveniles were detected at Sites 10 and 12, with no juveniles detected at Site 11, 42 days after release. No formal analysis was done to describe differences among treatments.

The proportion of juveniles in the outer strata (0.6 m) was greater than that closer to the point of release within the first two days, with the exception of that at the third site at each time (Sites 3 and 6; Figure 5.4). The mean proportion of juveniles within both strata combined (dashed line, Figure 5.4), within the first two days among sites, was ~5% of the 600 juveniles released at each site. Within the first two days of release 41% and 27% of juveniles remained within deployment devices, respectively. After 10 and 42 days the mean percent of juveniles surviving was 3.5% and 1.1%, respectively (dashed line, Figure 5.4), with <5% of the 600 released juveniles remaining within deployment devices from 10 days post-release.

Experiment 5

Within eight days of release, juveniles were detected between 5-10 m from their release point, with a portion of those individuals being close to the outer margin of this strata (Figure 5.5 and personal observation). A greater proportion of juveniles was detected on the substratum within 2 m of the release point at all locations and this was consistent through time. No juveniles were detected on the substratum beyond 2 m of the release point one day after release, or beyond 5 m of the release point four days after release. At each of the three locations, after eight days, the proportion of juveniles detected on the substratum among the four distance strata ranged between 8.6×10^{-2} and 0.8×10^{-2} of the ~5 500 juveniles released. After eight days of being released juveniles were more evenly distributed among the distance strata at each location, particularly within distance strata beyond 2 m from the point of release. The summed total of juveniles detected within all 4 distance strata within each of the three locations, eight days after release, represented between 9 and 13% of the ~5 500 juveniles released at each location.

5.3.4. Long-term sampling

Experiment 6

The density of juveniles 93 days post-release was greatest in samples where layers of boulders were disturbed (BQ) (Figure 5.6a). The mean density of juveniles were greatest in treatments

with high levels of habitat disturbance, i.e. BQ (Figure 5.6a, Table 5.5); an average of 49 abalone.m^{-2} were detected in the BQ treatment. Visual surveys (VQ) and the inspection of boulders *in situ* (B*is*) recovered 3% and 33% of samples taken where boulders were disturbed (BQ), respectively. Variation among replicate samples was generally low for all treatments, between 18-30% of the mean, and lowest within the treatment where boulders were disturbed (BQ).

The precision of samples obtained where boulders were disturbed (BQ) was greater than alternative methods at all levels of replication (Figure 5.6b). The standard error of samples obtained where boulders were disturbed represented an average of ≤0.2 of the mean where the sample size (*n*) was greater than 10 (Figure 5.6b). Similarly, the precision of samples obtained through the inspection of boulders *in situ* (B*is*) was greater than that where substrata was visually inspected without its disturbance (VQ) at all levels of replication (Figure 5.6b).

Experiment 7

The density of juveniles was greatest in the treatment where layers of boulders were disturbed (BQ) and this was consistent among locations (Figure 5.7, Table 5.6). Juveniles were detected on the substratum at all locations where the boulder disturbance (BQ) treatment was used and at two locations using visual surveys (VQ) (Figure 5.7). Visual surveys recovered less than 33% of samples using boulder disturbance, where juveniles were detected using both treatments. Variation among replicate samples was generally high for both the visual survey and disturbed boulder treatments, ranging between 61-100% and 31-61% of the mean, respectively.

Stratified sampling

Experiment 8

Densities of juveniles were consistently greater in the solid habitat treatment at all locations (Figure 5.8). Samples within the disturbed boulder field habitat recovered 15% and 29% of those samples from the solid habitat at Locations 1 and 2 respectively, at Area 2. No juveniles were detected in the disturbed boulder field habitat at Area 1. Variation among replicate samples was higher within the disturbed boulder field treatment at both locations at Area 2, ranging between 47% and 61% of the mean. Greater densities of juveniles detected at locations at Area 2 are likely due to a combination of factors including the greater numbers of juveniles released to each of these locations. No formal analysis was done to describe differences among treatments.

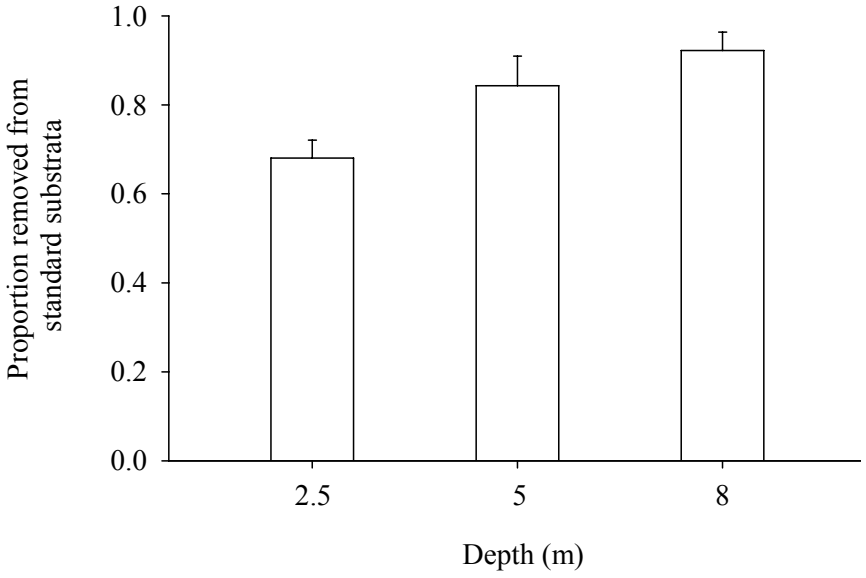


Figure 5.1 Experiment 1. Mean proportion of juveniles (+ SE) sampled from a standard substrata (paving block) at 3 depths using the venturi-lift (n = 5 blocks).

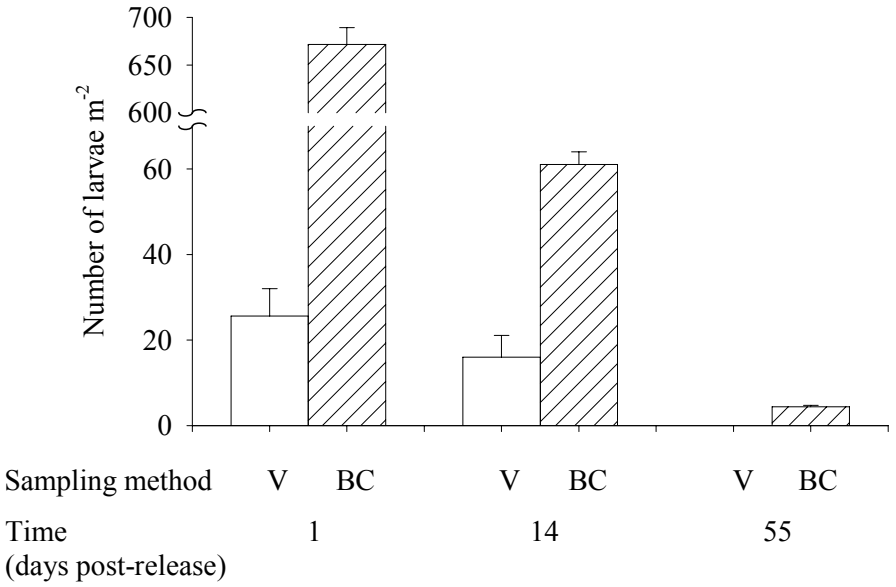


Figure 5.2 Experiment 2. Mean number of larvae.m^{-2} (+ SE) from samples taken using the venturi-lift (V) within 250 x 250 mm quadrats and the collection of boulders (BC), at one location 1, 14 and 55 days post-release (n = 5 quadrats and boulders).

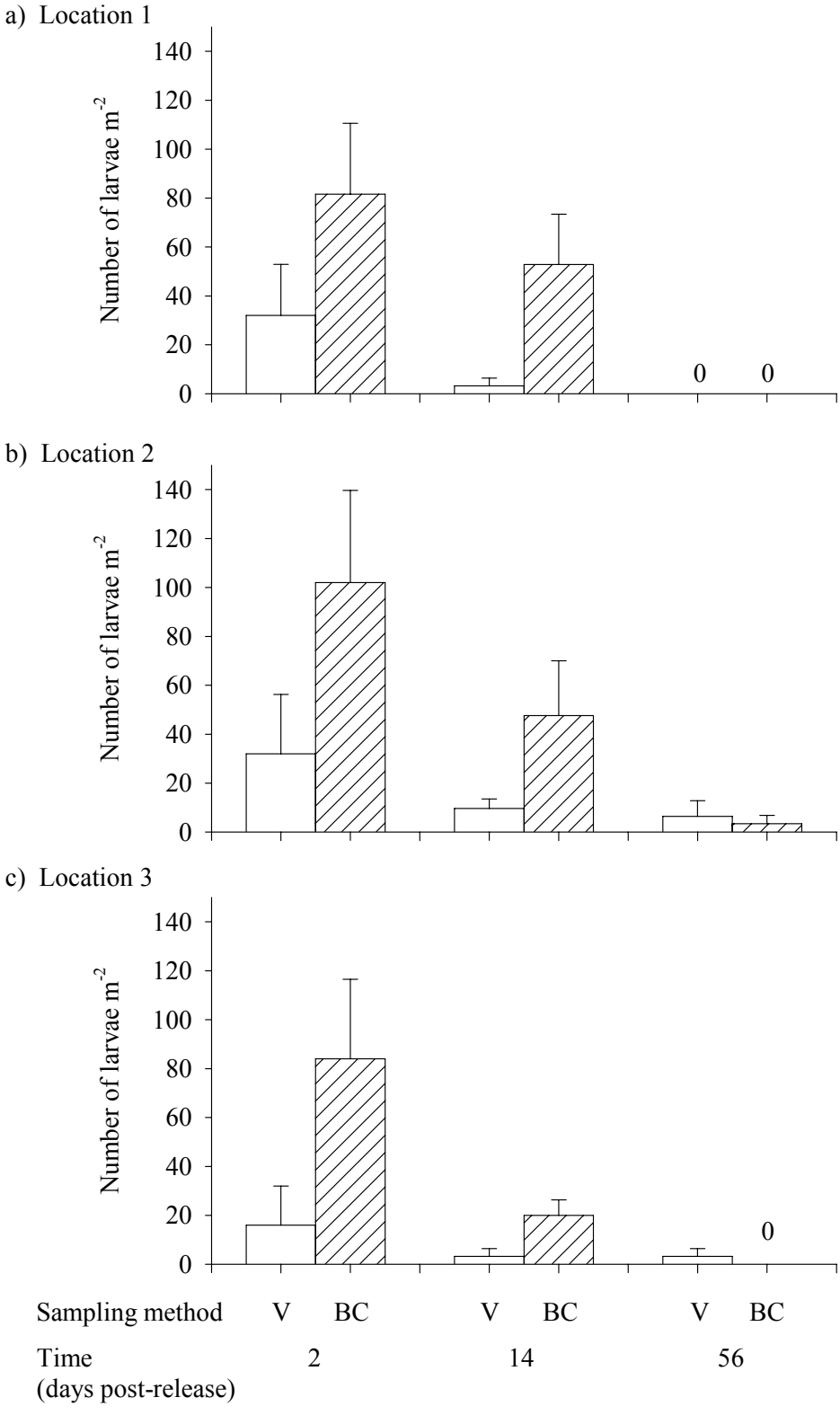


Figure 5.3 Experiment 3. Mean number of larvae.m^{-2} (+ SE) from samples taken using the venturi-lift (V) within 250 x 250 mm quadrats and the collection of boulders (BC), at three locations 2, 14 and 56 days post-release ($n = 5$ quadrats and boulders).

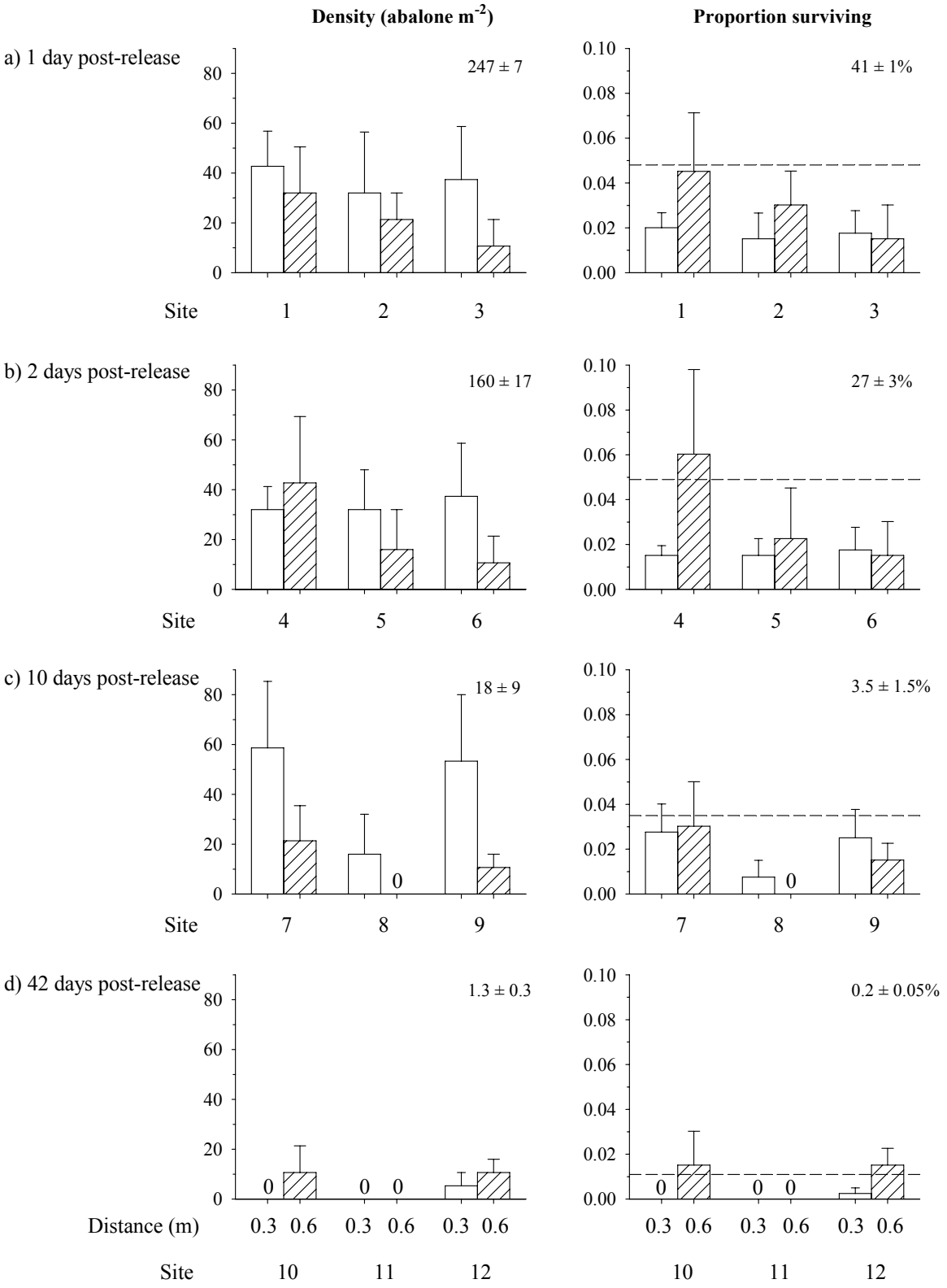


Figure 5.4 Experiment 4. Mean number of 2 mm juveniles.m^{-2} and proportion of total number surviving (+ SE), sampled by the venturi-lift within 300 mm, and between 300 - 600 mm of three sites, 1, 2, 10 and 42 days post-release ($n = 3$ quadrats). Dashed line indicates the mean total proportion of juveniles among sites at each time (calculated from the sum of both distance strata within each site). Numbers indicate the mean number and percentage (± SE) of the original 600 juveniles remaining on deployment devices at each time.

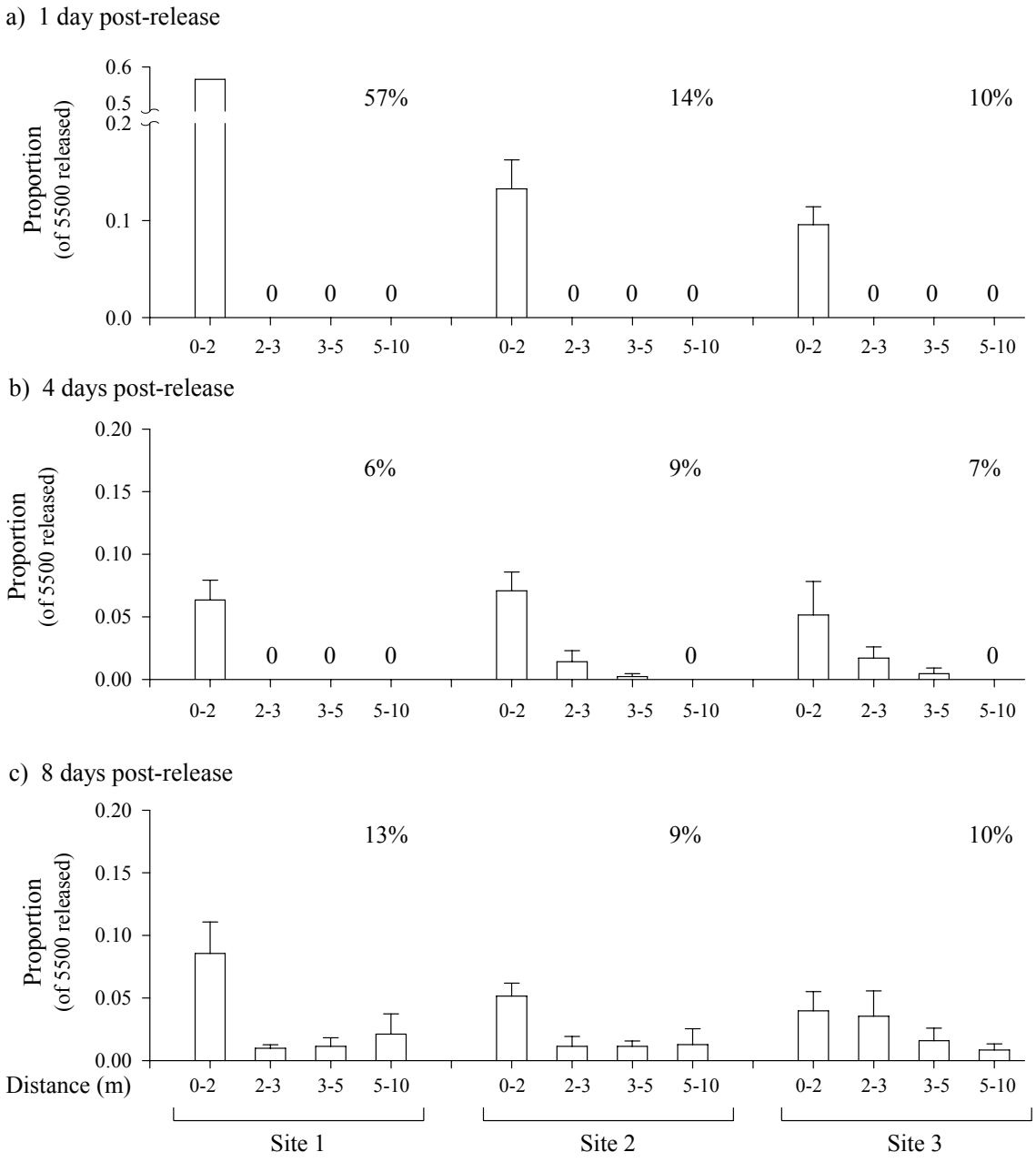


Figure 5.5 Experiment 5. Mean proportion (+ SE) of 11 mm juveniles surviving at three sites 1, 4 and 8 days post-release. Samples were taken using visual surveys within distance strata along transects (10 x 0.5 m) (n = 4 transects). Percentages represent the estimated total number of the ~5 500 released juveniles within each site at each time.

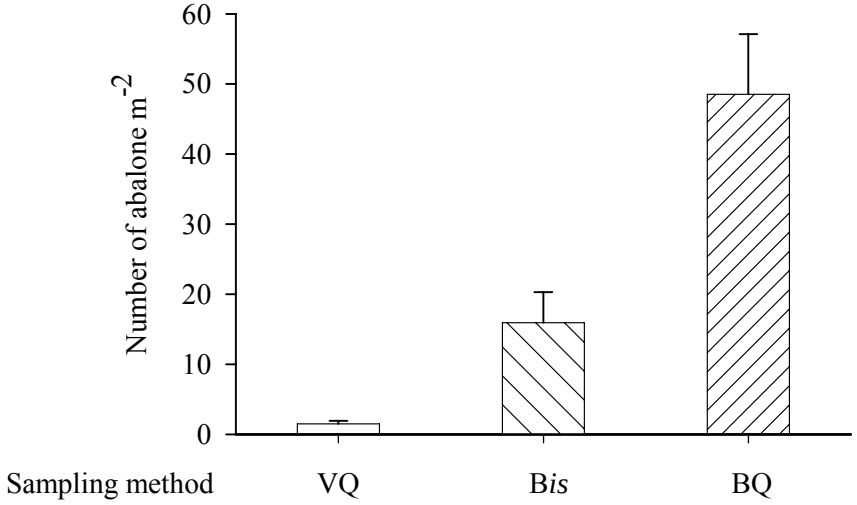


Figure 5.6a Experiment 6. Mean number of 14 mm juveniles.m^{-2} (+ SE) 93 days after release using three survey methods, visual quadrat (VQ), boulder inspection *in situ* (B*is*) and disturbed boulder quadrats (BQ), within 0.36 m^2 quadrats (n = 15 quadrats).

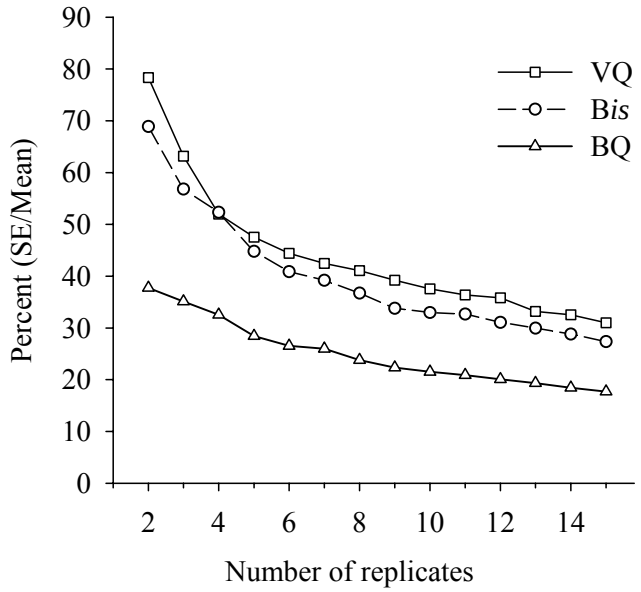


Figure 5.6b Experiment 6. Relationship between the precision (SE/Mean) of three sampling methods, visual survey (VQ), boulder inspection *in situ* (B*is*) and disturbed boulder sampling (BQ) and the number of 0.36 m^2 quadrats used to estimate the mean number of 14 mm juveniles.m^{-2}, 93 days post-release (n = 20).

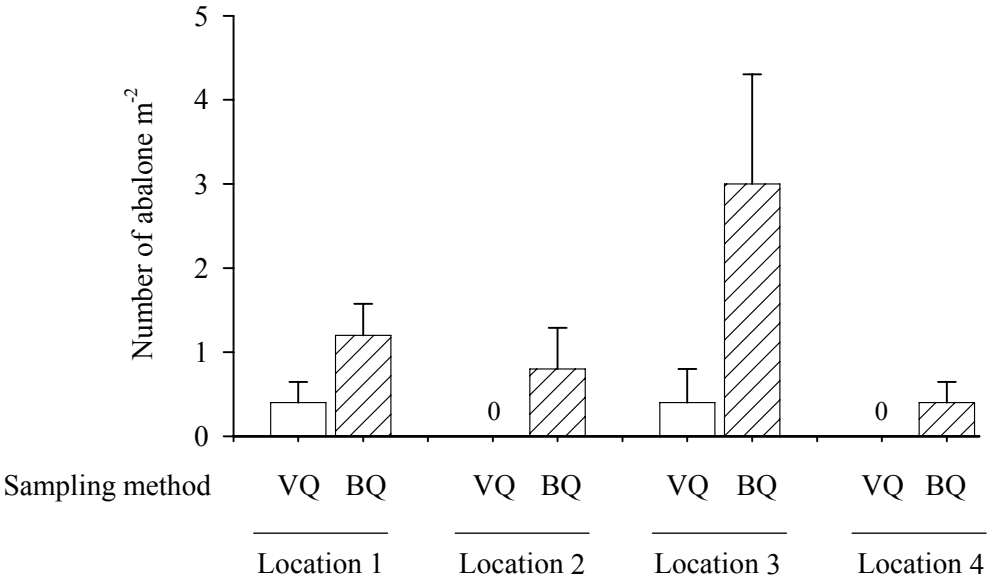


Figure 5.7 Experiment 7. Mean number of 40 mm juveniles.m^{-2} (+ SE) 122 days after release using visual surveys (VQ) and disturbed boulder quadrats (BQ) within 1 m^2 quadrats (n = 5 quadrats).

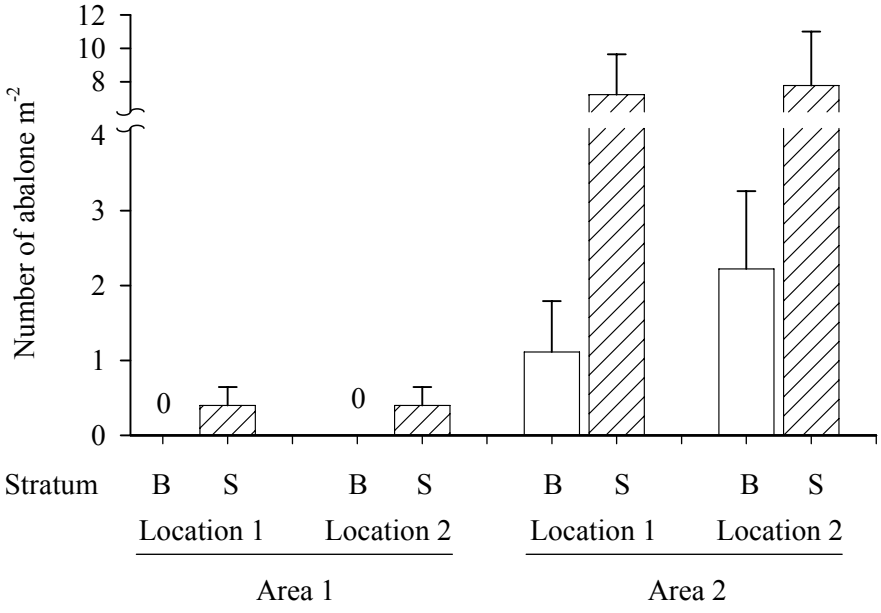


Figure 5.8 Experiment 8. Mean number of juveniles.m^{-2} (+ SE) ~770 days after release at two locations within two areas. Samples were taken in two stratum; boulder field habitat (B) and solid habitat (S), using disturbed boulder sampling within 1.0 and 0.36 m^2 quadrats at Areas 1 and 2, respectively (n = 5 quadrats).

Table 5.2 Experiment 1. Summary of analysis of variance in the number of 2 mm juveniles removed from a standard substrate at 3 depths using the venturi-lift ($n = 5$). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.5684$ ns; variances 3, df 4).

	Source	df	MS	*F*	F vs
1	Depth, D	2	0.076	5.90 *	2
2	Error	12	0.013		
3	Total	14			

Table 5.3 Experiment 2. Summary of analysis of variance in the number of abalone sampled.m^{-2} using the venturi-lift and boulder collection, 1, 14, and 55 days after their release as larvae at one location (n = 5). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.9716$*†; variances 6, df 4). †Square root (x + 1) transformed).

	Source	df	MS	*F*	F vs
1	Method, M	1	433.57	19.33 **	4
2	Time, T	2	465.81	20.76 **	4
3	M x T	2	256.66	11.44 **	4
4	Error	24	22.43		
5	Total	29			

Table 5.4 Experiment 3. Summary of analysis of variance in the number of abalone sampled.m^{-2} using the venturi-lift and boulder collection, 2, 14, and 56 days after their release as larvae at three locations ($n = 5$). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.1667$† ns; variances 18, df 4). †Square root (x + 1) transformed).

	Source	df	MS	*F*	F vs
1	Method, M	1	153.76	435.93 **	3
2	Time, T	2	172.33	119.40 **	6
3	M x T	2	51.10	14.56 *	7
4	Location, L	2	7.36	0.80 ns	8
5	M x L	2	0.35	0.04 ns	8
6	T x L	4	1.44	0.16 ns	8
7	M x T x L	4	3.51	0.38 ns	8
8	Error	72	9.22		
9	Total	89			

Table 5.5 Experiment 6. Summary of analysis of variance in the number of 14 mm juveniles.m^{-2} within 0.36 m^2 quadrats, sampled after 93 days using the sampling methods, visual inspection (VQ), boulders *in situ* (B*is*) and disturbed boulders (BQ) (n = 15). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 1.9391^{*\dagger}$; variances 3, df 14). †Square root (x + 1) transformed.

	Source	df	MS	*F*	F vs
1	Method	2	101.81	27.81 **	2
2	Error	42	3.66		
3	Total	44			

Table 5.6 Experiment 7. Summary of analysis of variance in the number of abalone sampled.m^{-2} within 1 m^2 quadrats at four locations using visual inspection (VQ) and disturbed boulders (BQ) (*n* = 5). Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.3910^{*\dagger}$; variances 8, df 4). †log (x + 1) transformed.

	Source	df	MS	*F*	F vs
1	Method, M	1	0.505	14.19 *	3
2	Location, L	3	0.117	2.75 ns	2
3	M x L	3	0.036	0.84 ns	2
4	Error	32	0.042		
5	Total	39			

5.4. DISCUSSION

The development of a monitoring strategy to accurately measure the abundance of released individuals through time is essential to measure the success of a release program (Caddy & Defeo 2003, Bell *et al.* 2005, Lorenzen 2005). This success may be measured by the contribution of survivors to support the stability and persistence of local populations or their recruitment to a fishery. The objective of this chapter was to develop a monitoring strategy to accurately estimate the abundance of released *H. rubra* within release locations. Methods investigated in this chapter enabled quantitative estimates of the abundance of *H. rubra* to be measured at points in time ranging from days and weeks to months and years, after their release to natural reefs as larvae or juveniles. Conclusions and recommendations for long-term monitoring of released abalone to natural reefs are provided (Section 5.4.4).

5.4.1. Monitoring released larvae

Methods used to monitor the settlement, establishment and early survival of released larvae were successful in measuring the abundance of settled larvae and early juveniles at release locations within at least the first 14 days. The collection of boulders provided greater and more precise estimates of the number of settled larvae and early juveniles than could be achieved using a venturi-lift, within 14 days of the release of larvae. Samples taken using a venturi-lift detected less than 32% of those using the collection of boulders within the same period of time.

The planar sample area described for samples taken using the venturi-lift (250 x 250 mm quadrat) regularly included areas of boulder habitat or bedrock that consisted of undulations and cracks such that the exposed areas of reef sampled by this method and the collection of boulders were comparable. That the areas sampled by each method could not be standardised, as described by McShane and Smith (1988), does not substantially detract from the differences in densities observed in this study. However, it is clear that the venturi-lift was most effective at sampling exposed substrata where its brush could effectively maintain contact with the reef. Boulder collections and the associated sampling of interstitial space supported a model for higher initial larval settlement and short-term survival on less exposed surfaces.

Only abalone less than the size of early juveniles (~2 mm) were reliably sampled using the venturi-lift in this study. This maximum length is similar to the results presented by McShane and Smith (1988, 1991), McShane (1991) and Nash *et al.* (1995) for wild *H. rubra* larvae. Further, the sampling efficiency of the venturi-lift increased with depth, with ~85% of 2 mm abalone sampled at 8 m. This is in contrast to claims by other authors that the venturi-lift could be used to sample all recruits of *H. rubra* up to 12 mm in length (McShane 1991, McShane & Smith 1991). This difference is possibly an artefact of the venturi-lift being used at slightly

greater depths by other authors (6-10 m reported by McShane and Smith (1991); thereby increasing its sampling efficiency). It is also possible that specific differences in the hardware and precise methodology could have contributed to these contrasting findings, as also described by Nash *et al.* (1995).

The complex boulder habitat and relatively shallow depth (<8 m) at locations could have limited the efficiency of these methods to accurately measure the abundance of released abalone, particularly at times after 14 days. This is possible as after 14 days early juveniles would likely have been more cryptic and their distribution more even throughout the topographically complex substrata, where there was greater than one layer of boulders. The small and inconsistent numbers of juveniles sampled after 55 and 56 days in Experiments 2 and 3, respectively, indicated high rates of mortality, limitations in the methods used to detect abalone estimated to be ~5 mm SL, or a combination of both of these and other factors affecting their abundance and distribution.

In contrast to other studies using a venturi-lift to survey settled *H. rubra* of similar size to those reported in this study, larvae that I collected were often damaged, and only positively identifiable as released individuals through observation of the fluorescent tag in the crushed larval shell. It is possible that numbers of settled abalone larvae in previously reported studies could have been under-reported due to the possibility of physical damage rendering a proportion of individuals in samples difficult or impossible to positively identify.

More precise estimates of the number of settled larvae were obtained where a greater number of larvae were released into a smaller area (Experiment 2), compared with estimates using the same sampling methods at locations where fewer larvae were released to a greater area (Experiment 3). It was possible that density-dependent effects influenced settlement, with larvae settling more consistently where space was more limited and in areas of less favoured substrata. Alternatively, preferred settlement substratum at the location in Experiment 2 may have been more uniformly distributed, resulting in reduced variation among samples. The identification of algal species preferred for larval settlement, and quantified estimates of their cover within larval release locations, would aid interpretation of the likely variation in abundance observed through time among future locations.

Alternative means of measuring short-term (<1 week) abalone larval settlement and survival could include the placement of artificial substrata within locations of interest (Davis 1995, Keesing *et al.* 1995, Nash *et al.* 1995, McShane & Naylor 1996, Kiyomoto *et al.* 2006). Pilot experiments within this study used conditioned settlement plates, evenly distributed throughout release locations prior to the release of larvae. Settlement of larvae on these plates was negligible and inconsistent (mean: 0 - 0.6 larvae.plate^{-1}, n = 5 plates). Where measures of

short-term larval settlement and survival are required to ensure the competency of larvae released to field locations, or to examine process likely to affect their initial settlement and survival, it is likely that settlement plates, inoculated with algal species known to provide high settlement preference (Daume *et al.* 1999, Kiyomoto *et al.* 2006), or other natural substrata (Huggett *et al.* 2005), could be used to provide more reliable and consistent measures. However, the cost of gaining short-term measures of settlement success is unlikely to provide substantial long-term benefit, particularly for large-scale releases of larvae.

Limitations of the venturi-lift demonstrated in results presented in this chapter (including its reduced effectiveness in shallow water, or as a consequence of the size of individuals being sampled), the potential to damage fragile structures (larval shell) and the effort required to obtain and sort samples does not support its continued use in the general monitoring of released larvae. Use of the venturi-lift to monitor released abalone would be best employed where alternate sampling methods are not possible, where sampling depth is >8 m and abalone are predicted to be <2 mm.

The cost effectiveness of accurately sampling field locations to measure initial settlement and early survival of released larvae is considerably affected by the small scale variation in settlement within locations and the labour intensive means of isolating and identifying newly settled larvae to be of hatchery origin in the laboratory. Even with the use of methods developed and described in this chapter to significantly reduce the time required to process samples (by up to 60%), and potentially complementary methods advocated by other authors (Hamilton 1969, McShane & Smith 1991), the costs of obtaining estimates of successful settlement and establishment of released larvae are substantial and opportunity costs are likely to be prohibitive. This is particularly the case for the monitoring of larvae released at large scales. It is possible that long-term sampling at locations where larvae are released could employ methods described for monitoring juveniles that would require substantially less investment in field and laboratory effort.

5.4.2. Monitoring released juveniles

Results from experiments in this study demonstrated that juveniles can move distances of up to 5-10 m within 8 days from their point of release, on natural reefs. The distance that juveniles disperse has important implications on defining the boundaries of a release location and the spatial scale of a monitoring strategy to assess their long-term survival. Rates of dispersal in this study manifest as a more homogeneous distribution of juveniles throughout the habitat surrounding the points of release within a relatively short period of time (8 days). This dispersal throughout the release location supported methods of long-term monitoring that assumed a homogeneous distribution of juveniles. Greater and more accurate estimates of the number of

juveniles surviving were detected using methods that disturbed the habitat within a release location. Experiments describing the dispersal of juveniles in habitat that could not be disturbed, and hence where sampling methods to obtain more accurate estimates of abundance could not be used, employed consistent methods among locations through time allowing relative estimates of abundance to be compared.

Juvenile dispersal

The potential of individuals within a population to move beyond the boundaries of a release and survey area can have significant implications on the accuracy of estimates of survival and hence the conclusions drawn from these data (Schwarz & Seber 1999, Schwarz 2005). The movement of abalone has been described by a number of authors in response to their ontogeny (Hines & Pearse 1982, Tegner & Butler 1989, Tarr 1995, Shepherd & Daume 1996, Kiyomoto & Yamasaki 1999) and levels of disturbance including that during the process of tagging (Poore 1972, Ault & DeMartini 1987, Day & Fleming 1992, Tarr 1995, Dixon *et al.* 1998, Guzman del Proo *et al.* 2004), habitat disturbance (Poore 1972), fishing (Dixon *et al.* 1998, Officer *et al.* 2001), food availability (Hines & Pearse 1982, Werner *et al.* 1995, Kiyomoto & Yamasaki 1999), density (Davis 1995, Tarr 1995) and release from a hatchery (Saito 1984, Tegner & Butler 1985, de Waal *et al.* 2003, Dixon *et al.* 2006). Distances moved by individuals reported in these studies range from <1 m and up to 100s m over timescales of ~1 month to 3 years.

Determining the scale of dispersal of juvenile abalone from their point of release is a critical component of developing a strategy to release individuals to natural reefs and to efficiently and accurately monitor their abundance as part of a stock enhancement program (Saito 1984, Tegner & Butler 1985, Tegner 1992, Schiel 1993, Werner *et al.* 1995, Tegner 2000, de Waal *et al.* 2003). That juveniles disperse into the surrounding habitat from a deployment device holding a large number of individuals is not unexpected. However, understanding the scales over which juveniles disperse can inform the structured release of aggregations of juveniles (in deployment devices) to sites within a release location so that the distribution of juveniles within the location becomes relatively even within a short period of time (Plate 5.4). The even distribution of juveniles within a release location can then support an efficient sampling design to accurately measure abundance whilst reducing negative affects of higher densities on survival e.g. pressure form roving predators. Moreover, a greater understanding of the scale of dispersal can inform the possible loss of individuals from a location due to migration and, where quantified, provide a means of providing less conservative, higher estimates of long-term survival, despite the fact not all survivors would be contributing from within a survey location (e.g. Glazer 2005). Habitat heterogeneity, behaviour and a number of other factors will ultimately influence the final pattern of distribution within and beyond a release location. However, the initial distribution of

juveniles within a release location and their dispersal does provide a more homogeneous distribution within a short period of time and supports more efficient sampling in the long-term.

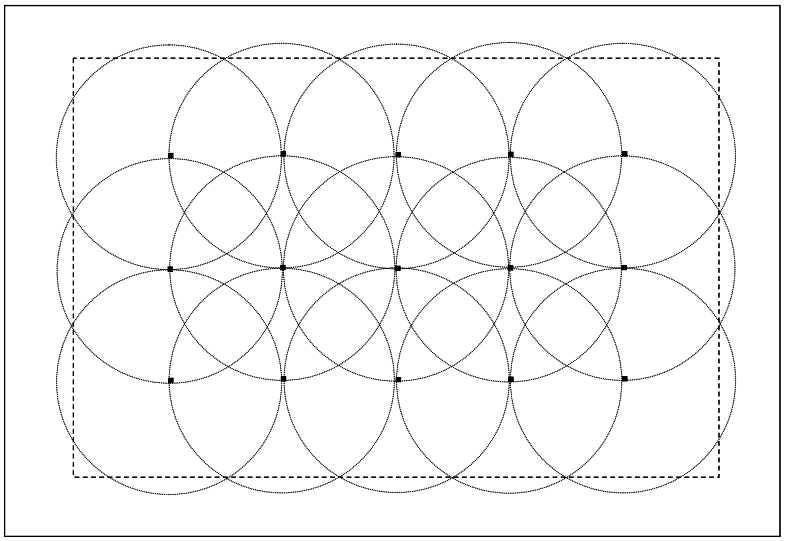


Plate 5.4 Representation of a release location (dashed box) containing a regular distribution of release sites (black squares), providing a distance of ~10 m between sites, allowing for the relatively even, theoretical, distribution of juveniles (circles) from a deployment device at each release site throughout the location after ~8 days, accepting a level of migration from the survey location into surrounding area (solid box), not to scale.

In this study hatchery-reared juveniles in experiments 4 (~2 mm SL) and 5 (11 mm SL) dispersed from their point of release up to 600 mm in one day and between 5-10 m in eight days, respectively. The rate of dispersal described for larger juveniles is similar to that described by Saito (1984) for *H. discus hannai* (8-50 mm SL) within 1-2 months. In contrast, de Waal *et al.* (2003) described the limited active dispersal (<3 m) of *H. midae* (12 and 24 mm SL) and significant differences in dispersal among locations, and suggested more active dispersal was due to increased exposure to intensive wave action. Locations in this study were on natural reef at a depth of ~6 m and were not subject to substantially different or extreme sea conditions during the survey periods. Moreover, the release locations provided an abundance of seemingly preferred habitat, including stable cryptic habitat and a diverse algal cover, suggesting a tendency for reduced dispersal activity (Tarr 1995, Dixon *et al.* 1998, de Waal *et al.* 2003, Dixon *et al.* 2006). Further, the disturbance of habitat and juveniles during sampling was negligible in each experiment and is unlikely to have contributed to the patterns of dispersal. For example, in Experiment 4 each site was sampled only once, and sampling in Experiment 5 consisted of visual surveys, where no habitat or individuals were disturbed. However, relatively high initial densities of juveniles (Davis 1995) and associated factors including reduced food

availability (Werner *et al.* 1995) in concert with processes involved in handling and release (Saito 1984) could have resulted in a greater tendency for active dispersal.

The scale of the distance strata surveyed to monitor the dispersal of larger juveniles (11 mm SL) was adequate to detect dispersal within the time periods surveyed. This was demonstrated by no juveniles being detected beyond 2 and 5 metres after 1 and 4 days, respectively and the detection of juveniles in this more distant strata after 8 days. Further, that the spatial distribution of larger juveniles became relatively consistent among the distance strata through time, beyond 2 m from their point of release, indicated reduced active dispersal over the same period. These results support the conclusion that an array of release sites separated by up to 5-10 m within a location can result in the substantial overlap of abalone dispersed from different sites within a short period of time, and can facilitate sampling within the location to obtain representative estimates of density and monitoring of long-term survival.

Despite results from Experiment 4 and 5 indicating that the methods used were adequate to describe juvenile dispersal and provide relative measures of abundance through time, the total number of abalone detected within sites at any time indicated that >50% and >80% of those juveniles released were unaccounted for, respectively (with the exception of Site 1, after 1 day in Experiment 5). Further, results from both experiments indicate that it was unlikely that migration from the survey area was a substantial contributing factor in these findings. For example, in Experiment 4 there were reliable averages of 41%, 27% and <5% of juveniles remaining on deployment devices after 1, 2 and 10 days, respectively; small proportions (<5%) of juveniles were detected within both distance strata from the release point and; <3% of juveniles were detected in the most distant strata (600 mm) at any time. This suggests that migration through the most distant strata was unlikely to have substantially contributed to the 50% of juveniles unaccounted for and indicates the importance of devices to act as a source of juveniles. In Experiment 5, 6 - 14% of the 5500 juveniles released to each site were detected at any time (with the exception of Site 1, one day after release) and the dispersal distances surveyed were demonstrated to adequately detect dispersal during the survey period. However, it is likely that migration from the release location could occur during longer periods of time. For example, Saito (1984) described the final uniform dispersal of released *H. discus hannai* (8-50 mm SL) after 1 year, over an area of 50 m from their release point. Extending the sampling effort beyond the immediate release area to obtain quantitative estimates of the abundance of released individuals was not possible within the resource constraints of this thesis. However, an assessment of the contribution and impact released individuals could have beyond the immediate release area would require consideration particularly where any large-scale release of individuals was planned.

It was concluded that the sampling methods used to assess dispersal were inaccurate at sampling absolute abundance. This is likely to have been due to topographically complex and solid habitat that could not be disturbed for sampling, and the behaviour of released juveniles (to seek more cryptic habitat). Other processes likely to have affected estimates of the absolute abundance include predation (Hines & Pearse 1982, Tegner & Butler 1985, 1989, Shepherd *et al.* 2000, Dixon *et al.* 2006) and mortality associated with the process of handling and release, particularly within short periods of time after release (Tegner & Butler 1989, Schiel 1993, McCormick *et al.* 1994). Predation of hatchery-reared and released abalone can be high (Tegner & Butler 1985, McCormick *et al.* 1994, Shepherd *et al.* 2000). Tegner and Butler (1985) describe annual estimates of mortality, measured as the percent of recaptured shells within a release location, of up to 42%. Further, through investigation of the type of damage to the recovered shell, they attributed equal proportions of mortality to predation by octopus, predation by crustaceans and processes related to handling and release. However, accurately estimating mortality associated with predation, through the collection of discarded shells and typing of shell damage, is problematic. This is not only because some predators may destroy the shell beyond recognition (Schiel & Welden 1987) but also because some predators, including fishes, may consume the whole animal, leaving no evidence in the form of a discarded shell (McCormick *et al.* 1994, Shepherd & Clarkson 2001). However, it was concluded that processes associated with mortality were unlikely to have made a relatively substantial contribution to the large underestimates of absolute abundance, compared with that of the complex topography combined with the behaviour of juveniles to seek cryptic habitat. This is because there was no indication of high mortality of abalone within 24 hours of release. For example, there was no observation of high numbers of empty or broken shells or high rates of intensive predation within short periods of time after release (personal observation).

5.4.3. Long-term sampling

The abundance of juvenile and early adult haliotids, particularly *H. rubra*, is difficult to accurately assess because of their cryptic distribution often among complex topography in rocky habitat. The physical attributes of this habitat restrict the methods available to carry out assessments within it. Habitat unable to be disturbed and thoroughly sampled, including that consisting of boulders cemented together by a cover of encrusting algae and concreted sediment, and bedrock with cracks and fissures, provides many niche areas similar to those provided by similar habitats that can be disturbed, such as boulder fields. Methods to obtain the most accurate estimates of the abundance of juveniles and early adults requires samples to be taken from the entire habitat supporting their distribution. To determine the best methods for assessing

long-term abundance of released juveniles, estimates of abundance were compared among methods employing different levels of habitat disturbance within boulder fields.

I demonstrated that different methods provide significantly different measures in the accuracy and precision of samples to estimate density, and consequently abundance over long periods. Disturbance of the substrata was a key issue. Samples obtained where substrata was disturbed provided greater, more precise and more accurate estimates of the density of juveniles released. Boulder sampling *in situ* detected about 33% of samples where boulders were disturbed but provided consistently greater estimates of the density of juveniles than visual surveys, whilst only minimally disturbing the habitat and the abalone within it. Visual surveys, where no substrata was disturbed, detected 0-5% and consistently <33% of juveniles where boulders were disturbed. Visual surveys provided the lowest precision of each of the methods tested and Boulder sampling *in situ* provided measures of precision similar to those of visual surveys.

The relative imprecision and inaccurately low estimates of density obtained using visual surveys and boulder sampling *in situ* indicates these methods are unlikely to provide rigorous data to assess the success of releasing abalone to natural reefs. However, that abalone become less cryptic as they become larger suggests it is possible that visual surveys may support measures of abundance after longer periods of time that are more precise and accurate than those provided within months of release. This is more likely to be the case where surveys are done in habitat that is not topographically complex and where abalone have grown to a length where they become emergent. It is also likely that visual surveys can provide adequate measures of relative abundance (Andrew *et al.* 1996, Hart *et al.* 1997, Gorfine *et al.* 1998, Tarr *et al.* 2000, Mundy *et al.* 2006), particularly of emergent and adult abalone populations.

Thorough sampling of boulder habitat, including multiple layers of boulders, provided the greatest, most precise and accurate measures of abalone density. In particular, where abalone had been released for >3 months, this method consistently detected the presence of abalone where methods employing visual surveys did not (Experiment 7). That more reliable and consistent estimates of the number of abalone surviving though time could be obtained using sampling methods that employed increasing levels of disturbance of the substratum was not surprising, considering the niche cryptic habitats preferred by the different life history stages of *H. rubra*.

Where boulder field habitat within release locations had been substantially disturbed by natural processes (rough seas) after the release of individuals, long-term measures of abalone abundance can be more precisely and accurately determined where a stratified sampling approach is used to account for observed unequal densities among two physically defined areas. For example, where juveniles had been released to natural reefs for >2 years and where release

locations had been exposed to episodes of habitat disturbance caused by rough seas, samples taken from disturbed boulder habitat recovered <30% of samples taken in more solid habitat (Experiment 8).

Greater numbers of smaller replicates provided greater levels of precision in the density of abalone detected at a release location, although comparative samples were not obtained from the same locations (Experiments 6 and 7). Despite this, the precision of a number of methods was low, reflecting the small scale patchy distribution of abalone within locations as well as the number of replicate samples taken. Moreover, larger sample units were often required to obtain measurements of density using the stratified sampling approach as boulders were required to be located within a quadrat that also contained between 25 - 50% of solid habitat (Section 5.2.5, Stratified sampling). Juveniles were conspicuous and sampling was undertaken by divers that were experienced and trained to observe small abalone underwater. Further, sampling was generally undertaken during calm conditions with good underwater visibility. It is unlikely that observer error could have substantially affected differences among times or between the methods tested.

5.4.4. Conclusion and recommendations

A number of methods used to measure the abundance of settled larvae and juvenile abalone indicated that accuracy and precision is affected by a number of factors including depth, size of individuals and habitat type in addition to the specific sampling method and level of replication. That sampling methods that did not disturb the substrata returned less precise, less accurate and lower estimates of the numbers of individuals surviving meant that the potential effects of sampling disturbance on successive estimates were required to be accepted as part of the monitoring strategy to estimate the abundance of abalone released as larvae or juveniles to natural reefs though time. Importantly, as most factors affecting the accuracy of sampling methods resulted in underestimates of recaptured abalone and that only positively identified individuals were assessed, estimates of survival derived from these methods are likely to be conservative and constitute minimum values.

Sampling released larvae within the period of time before they grow to early adults can be prohibitively costly in terms of field sampling and laboratory sorting time. Further, the use of the venturi-lift described in this study to measure larval settlement and persistence through to early juveniles (~2 mm) has limitations including depth related inefficiencies. Use of this method in shallow water and on individuals estimated to be in excess of ~2 mm would likely provide results biased towards an underestimation of abundance. Where the abundance of individuals sourced from the release of larvae are sought within timeframes of 0-3 months, sampling via the collection of boulders is likely to provide more precise and accurate estimates.

The monitoring strategy to measure the abundance of abalone released as larvae in Chapter 6 employed boulder collections within the first 2 months after release.

Investigation of the dispersal of juveniles released among complex habitat was critical for subsequent releases (Chapter 6). Juveniles, released in deployment devices to sites separated by up to 5-10 m within a release location, can disperse into the surrounding habitat and become relatively evenly distributed within relatively short periods of time (<1 month). This distribution of juveniles supports efficient sampling designs involving the haphazard placement of sample units within a release location to obtain representative estimates of the number of juveniles surviving rather than having to stratify the survey area by distance from each release site.

Disturbed boulder sampling within boulder field habitat resulted in more precise and accurate estimates of abundance of released juveniles through time compared with alternative methods tested. Further, sampling effort enabling greater numbers of smaller replicates resulted in more precise estimates of density for equivalent effort. However, where boulder habitat within release locations showed signs of substantial disturbance, indicated by boulders being rumbled clean of algae or with algal growth on the underside of boulders, stratified sampling within the boulder field and solid habitat would be necessary to provide more accurate estimates of the number of released individuals surviving. Long-term sampling within release locations should employ methods enabling high levels of replication and support the disturbance of the substrata so that the habitat supporting the population can be thoroughly sampled.

Using sampling methods that enable the most accurate measures of the abundance of released juveniles to be made over long periods of time (~18 months - 2 years), will allow the utility of releasing *H. rubra* to enhance local populations to be determined. Further, tagged individuals recaptured after long periods of time will enable estimates of the rate of growth to be included in assessing the bio-economic feasibility of releasing juveniles. In conclusion, multiple approaches are required if assessments of abundance are required from the settlement of larvae in the field to abalone of commercial length. Most assessments may more simply focus on the release of larger individuals and thus their assessment only requires a sampling strategy where individuals can be quickly and easily identified in the field using sampling methods that thoroughly survey the habitat supporting them. The indelible tagging of released individuals (Chapter 3) is critical for their identification throughout the assessment process. The results of long-term releases and a bio-economic assessment of a stock enhancement program for *H. rubra* in NSW are presented in Chapter 6.

6. LONG-TERM SURVIVAL AND GROWTH OF RELEASED *HALIOTIS RUBRA* AND A BIO-ECONOMIC ASSESSMENT OF ITS STOCK ENHANCEMENT IN NSW

6.1. INTRODUCTION

The primary objective of a stock enhancement program is to add value to a population, either through the contribution of individuals to support its viability or to support the harvest of released individuals and provide positive economic returns (Hilborn 1998, 1999, Bell *et al.* 2005, Lorenzen 2005, Bell *et al.* 2008). Estimates of long-term survival and growth of released individuals are critical to assess this objective, and form the basis of the biological component in a bio-economic assessment of such a program to determine its financial viability. Further, the impact of releasing individuals on the abundance of the wild population is an important factor in determining the value of a release program. If released individuals replace, rather than supplement, processes that enable the growth of a wild population, the value of a release program is likely to be negligible (Hilborn 1998, Bell *et al.* 2005).

Spatial and temporal variation in demographic processes affecting the population size of many marine organisms are well documented (Keough & Downes 1982, Olafsson *et al.* 1994, Caley *et al.* 1996, Eckman 1996). Further, depensatory processes affecting population size can have significant consequences on the persistence of a local population (Myers *et al.* 1995, Dulvy *et al.* 2003, Gardmark *et al.* 2003). Abalone exhibit demographic characteristics that make local populations vulnerable to depensatory effects, over exploitation and decline (Prince *et al.* 1987, Prince *et al.* 1988a, Babcock & Keesing 1999). The long-term persistence of ecologically functional abalone populations at spatial scales where abundance has declined due to processes such as disease and overfishing has not been formally assessed (although see Hobday *et al.* 2001, Miner *et al.* 2006). However, the loss or decline of abalone populations that have supported more viable commercial fisheries has been widely described (Breen 1986, Guzman del Proo 1992, Haaker *et al.* 1992, Tegner *et al.* 1992, Masuda & Tsukamoto 1998, Shepherd *et al.* 2001, Branch & Clark 2006, Appleford *et al.* 2008). The decline in abundance of local populations of *H. rubra* in NSW, Australia has been wide spread with declines of over 75% of historical levels in areas at the northern expanse of the fishery, due in substantial part to disease (Anon 2005c). There has been limited recruitment to these populations after declines were observed up to eight years previously, with the populations continuing to reflect levels of substantial depletion (Anon 2005c, 2008b).

Research into the use of hatchery-reared abalone to enhance local populations has a history dating back to the 1960s (Ino 1966, cited in Tegner & Butler 1985, Gutierrez-Gonzalez & Perez-Enriquez 2005, Tauchi 1984, cited in Hamasaki & Kitada 2008). Since then, the release

of abalone, primarily as juveniles, has been trialled at numerous scales in many countries including Australia (Preece *et al.* 1997, Shepherd *et al.* 2000, Dixon *et al.* 2006, Goodsell *et al.* 2006, James *et al.* 2007), Japan (Saito 1984, Kojima 1995, Masuda & Tsukamoto 1998, Seki & Taniguchi 2000, Hamasaki & Kitada 2008), Mexico (Guzman del Proo *et al.* 2004, Gutierrez-Gonzalez & Perez-Enriquez 2005), New Zealand (Schiel 1992, 1993, Roberts *et al.* 2007b), South Africa (Sweijd *et al.* 1998, de Waal *et al.* 1999) and the United States of America (Tegner & Butler 1985, 1989, Ebert & Ebert 1991, Rogers-Bennett & Pearse 1998, Tegner 2000). These studies have demonstrated substantial differences among species, along with spatial and temporal variation in success; as measured by the proportion of released individuals surviving through time. This variability highlights the difficulty and risk in developing and applying generalities across species and scales (see summary tables in McCormick *et al.* 1994, Shepherd 1998, Stoner & Glazer 1998, Bell *et al.* 2005, Hamasaki & Kitada 2008). The commercial-scale release of abalone has only been supported and continued in Japan, where between 6.9-83.5% of the abalone released at local scales have been recaptured to the commercial fishery (Hamasaki & Kitada 2008). However, review of these expansive release programs, that have continued over recent decades, highlight significant spatial variation in value, dominated by variation in survival, and a need for rationalisation to improve efficiencies and effectiveness (Masuda & Tsukamoto 1998, Seki & Taniguchi 2000, Hamasaki & Kitada 2008).

The release of larvae as a means to enhance local populations of abalone has been investigated at small scales and at few locations (Tegner *et al.* 1986, Tong *et al.* 1987, Schiel 1992, Preece *et al.* 1997, Shepherd *et al.* 2000, Tegner 2000). The motivations for these investigations have predominately included the relatively low cost of producing larvae (Schiel 1992, Tegner 2000, Gutierrez-Gonzalez & Perez-Enriquez 2005), failure of juvenile releases and recruitment limitation (Tegner *et al.* 1986, Tegner 2000) and the possibility of increased fitness of surviving individuals compared with hatchery-reared and released juveniles (Tong *et al.* 1987). However, despite rates of survival reported by Tong *et al.* (1987; ~0.4% at 2-3 mm) and Schiel (1992; 0.06% at 4-5 months, ~5 mm), the release of abalone larvae has been described as of limited value, compared with the release of juveniles. This has been due to the interaction of a number of factors including comparative value, high rates of mortality, proposed logistic difficulties in their release and increased uncertainty of results (Schiel 1992, Shepherd *et al.* 2000). Despite this, the practice has continued, without quantitative assessment, in Mexico since the 1960s and regularly since the 1980s (Gutierrez-Gonzalez & Perez-Enriquez 2005). There has been no long-term (>18 months) quantitative assessment of the contribution released, batch-tagged larvae may provide to enhance local populations of abalone.

Abalone released on to natural reef suffer high rates of mortality soon after release (Tegner & Butler 1985, 1989, Schiel 1992, Osumi 1999, Shepherd *et al.* 2000, Tegner 2000). Some of the

proposed mechanisms by which this occurs have included disproportionately higher rates of predation and/or competition for limiting resources where they have been released in dense aggregations within small areas (Tegner & Butler 1989, Shepherd *et al.* 2000, Tegner 2000, Heasman 2006). However, a number of studies have described relatively high rates of survival of juvenile abalone released at high densities within small areas (McCormick *et al.* 1994, Sweijd *et al.* 1998, Seki & Taniguchi 2000, de Waal & Cook 2001, Roberts *et al.* 2007b). It is likely that where dense aggregations of juvenile abalone have been released at small spatial scales, rates of dispersal (this study; Chapter 5, Saito 1984, Seki & Taniguchi 2000, de Waal *et al.* 2003) may negate or limit processes driving potential differences in mortality associated with high release densities. That juvenile abalone released in fewer dense aggregations may suffer higher rates of mortality than if released in more aggregations of lower density has obvious and important implications for initial and long-term estimates of survival, as well as the logistic management of abalone through the process of their release and therefore the value of a stock enhancement program.

The rates of survival of released juvenile abalone have also been described as being substantially affected by their length at release, with rates of survival increasing with length, although spatial variation among rates of survival is high (Saito 1984, Inoue 1976, cited in Tegner & Butler 1989, Roberts *et al.* 2007b). There are also several studies where the length at release of juvenile abalone has had no effect on survival, commonly where survival has been very low among all length classes (Tegner & Butler 1985, 1989), or where differences in survival have been primarily attributed to either disturbance (Schiel 1993) or differences (Seki & Taniguchi 2000) among habitats. Schiel (1992) also describes the increased likelihood of larger abalone being exposed to detrimental conditions within a hatchery rendering them more susceptible to mortality once released. Further, Seki and Taniguchi (2000) and Roberts *et al.* (2007b) describe substantially increased value in the release of juveniles <30 mm and ~10 mm, respectively, into specifically defined habitats. The length of hatchery-reared abalone has important implications for the value of a release program, including the costs of production, because larger abalone cost more to produce than smaller abalone. Costs associated with larger individuals include production limiting costs such as reduced space to grow individuals and greater financial inputs of food and maintenance for longer periods of time. Although it is generally accepted that increases in size at release are commensurate with increased rates of survival, there is a point where production costs outweigh likely returns from improved rates of survival (Tegner & Butler 1989, Schiel 1992, Roberts *et al.* 2007b, Hamasaki & Kitada 2008). The lengths of *H. rubra* juveniles (7-15 mm SL) used in this study represent lengths at which instantaneous rates of natural mortality decline rapidly from ~2.5 (Shepherd & Breen 1992) and

lengths at which rates of production within a hatchery allow substantial numbers of individuals to be produced.

Rates of growth of released abalone are important as they, in association with estimates of survival, enable estimates of the time required for released abalone to contribute to important demographic (maturity) and fishery (minimum legal length; MLL) length-classes in a local population. Further, faster growing abalone will survive at greater rates where length-related processes affecting survival operate. High rates of growth and other demographic processes that interact to result in a more productive population have important implications for the value of a release program. Greater rates of growth and survival translate to greater proportions of larger length classes and greater financial returns as more abalone reach harvestable size within shorter time periods.

Despite the importance of assessing the impact of releasing individuals on wild populations and the consequent effect on the value of a release program (Hilborn 1998, Caddy & Defeo 2003, Hilborn 2004, Bell *et al.* 2005), few studies within the marine environment describe any assessment of wild populations in association with releasing individuals (but see Leber *et al.* 1995, Crowe *et al.* 2002, Seitz *et al.* 2008). Unless appropriate resources such as food and space are available for released individuals and/or the natural population is not recruitment limited, it is unlikely that released individuals will provide a net increase to population growth or future harvests (Hilborn 1998, Caddy & Defeo 2003, Hilborn 2004, Bell *et al.* 2005). In fact, the release of individuals may precipitate negative impacts on wild populations through increased pressure on natural resources thereby limiting the growth of the wild population, increasing levels of predation through the attraction of itinerant predators, replacement of natural recruits and reduced genetic integrity of future populations. There have been no reported studies describing the monitoring of wild and released individuals at multiple release and control locations to assess the long-term impact of releasing individuals on the abundance of wild populations.

Following from this, the value of many stock enhancement programs has been criticised for a lack of accountability and the determination of targets to facilitate assessment (Hilborn 1998, Caddy & Defeo 2003). Bio-economic assessment and determination of investment choices will be intrinsically tied to the management objectives of a stock enhancement program (Lorenzen 2005) as well as the management policy developed to control and guide the returns to investors and stakeholders (Arnason 2008).

Important drivers of any bio-economic assessment of a stock enhancement program are accurate estimates of biological parameters including survival and growth of released individuals in association with estimates of economic inputs and returns. Further, the decision to invest in a

stock enhancement program requires an understanding of the biological system being manipulated and the financial capacity of the fishery. The nature of the population depletion is an issue with respect to the projected and realised success of an enhancement program. Populations depleted through disease are likely to result in lower rates of enhancement success than those depleted through overfishing. Finally, investment in a financially viable stock enhancement program requires comparison against alternative investment opportunities. A means of assessing this is through analysis of the Net Present Value (NPV) of the investment against the opportunity cost of an alternative, providing a declared rate of return (Caddy & Defeo 2003).

The objectives discussed in this chapter were to: 1) quantify the long-term (>2 years) survival and growth of batch-tagged *H. rubra* larvae and juveniles released to natural reefs; 2) determine the impact of releasing *H. rubra* on the abundance of wild populations; 3) provide a bio-economic analysis of a stock enhancement strategy for *H. rubra* in NSW; and 4) provide recommendations for the implementation of a stock enhancement management strategy for the NSW abalone fishery.

A series of six field experiments were done to determine: a) the long-term (>2 years) survival and growth of abalone, released as larvae, to replicated locations; b) the effect of releasing juveniles in a diffuse array or as a concentrated group on their long-term (>2 years) survival and growth; c) the impact of releasing juveniles on the abundance of the total and wild abalone populations at multiple locations, through the monitoring of released and wild abalone at release and control locations through time; d) the long-term (>2 years) survival and growth of abalone, released as juveniles, to replicated and individual locations across the geographic distribution of the NSW abalone fishery; and e) provide data to support a bio-economic assessment of the financial viability of an abalone stock enhancement program.

6.2. METHODS

All measures of abalone survival and growth described in this chapter are derived from the recapture of hatchery-reared, batch-tagged and released abalone larvae and juveniles using the methods and recommendations described in Chapters 4 and 5, that are summarised in sections 4.4.3 (Chapter 4) and 5.4.4 (Chapter 5), respectively and in Table 6.1. All releases and the monitoring of released and wild abalone populations were done at locations consisting of areas of natural reef that had not been manipulated or disturbed prior to sampling. Locations extended from Fingal Island, Port Stephens, on the lower north coast to Disaster Bay on the south coast of New South Wales, Australia (Figure 6.1). Reference to larvae, juveniles and abalone throughout this chapter refers to those that have been batch-tagged and positively identified as of hatchery origin, unless otherwise stated in the text. The term 'survival' is used conservatively in the context of the total number of positively identified abalone recovered during sampling from within a defined release location only and therefore constitutes minimum survival rates. General protocols describing location selection criteria, the general sampling strategy, including that within solid habitat are outlined below in Section 6.2.4.

6.2.1. Long-term survival and growth of larvae

Experiment 1 - survival and growth of released larvae

Experiment one was designed to measure the spatial and temporal variation in survival of released larvae. The shell length of recaptured abalone was measured to describe variation in the rates of growth among individuals released as larvae. It was predicted that the total number of abalone within locations would decline rapidly within the first 60 days post-release and that survival of abalone through time would be consistent among locations.

At each of three locations (A, B and C) along the north coast of Fingal Island, Port Stephens, NSW (Figure 6.1; Section 6.2.4.1 Location selection) 1.1 million larvae were released. Locations were sampled at 7 times up to 553 days post-release (1.5 years; Table 6.1, Section 6.2.4.2 Sampling strategy). Sampling methods included the collection of boulders (BC), boulder sampling *in situ* (B*is*) and boulder sampling with disturbance (BQ; Chapter 5, Section 5.2.5; levels of replication and sample unit size are detailed in Table 6.1). All abalone that were sampled were collected and examined for positive identification of a calcein labelled, batch-tagged, larval shell (as described in Chapter 3, Section 3.2.1). All positively identified abalone collected at 196 and 553 days post-release had their total shell length (SL mm) measured (Table 6.1; Section 6.2.4.3 Growth).

An additional 2 million larvae were mistakenly added to each location 237 days after the initial release. The differentiation between abalone sampled 553 days after the initial release with those released 237 days later was determined by inferring the rates of growth required to reach the length of those individuals recaptured. All of those positively identified abalone recaptured 553 days after the initial release were allocated to have survived from the initial release. This decision was based on the improbable growth rates of between 110 - 180 μm.day^{-1} (average 130 μm.day^{-1}) required if they had originated from those larvae released 237 days after the initial release.

6.2.2. Long-term survival and growth of juveniles

6.2.2.1. Replicated locations

Experiment 2 - diffuse and concentrated release of juveniles

Experiment two was designed to test the hypothesis that the number of juveniles surviving after >2 years at locations where they were released in a diffuse array (10 deployment devices, hereafter called devices, of 100 juveniles) would not be significantly different from the number surviving at locations where they were released in a concentrated group (one device holding 1 000 juveniles). Further, it was hypothesised that there would be no significant difference in the mean rate of growth (mm.yr^{-1}) of abalone released in a diffuse array or in a concentrated group after >2 years.

Six locations were defined along the northern coast of Disaster Bay, NSW (Figure 6.1; Section 6.2.4.1 Location selection). At each location 1 000 abalone (15 mm SL) were released either in a diffuse array of devices (Locations A-C) or a single concentrated group (Locations D-F; Table 6.1). Within 'diffuse' locations the 10 devices were evenly distributed among the habitat within an area of ~115 m^2. Where abalone were released to 'concentrated' locations a single device was placed at the centre of an area defined by a 6 m radius (113 m^2).

Within the first 363 days, sampling within 'diffuse' locations consisted of disturbed boulder sampling within quadrats haphazardly distributed throughout each location at each time (n = 25; Table 6.1; Section 6.2.4.2 Sampling strategy). Similarly, sampling within 'concentrated' locations, within the first 363 days, utilised the same total number of quadrats and disturbed boulder sampling method. However, sampling at 'concentrated' locations was done within specific distance strata from the device so that concentric rings of area radiating from the centre of the site were sampled in equal proportion. The distance strata were increments of 0-2, 2-3, 3-5 and 5-6 m from the release point. The number of quadrats sampled within each stratum was 3, 3, 11 and 8 respectively. Quadrats were haphazardly distributed within each stratum at each location. This sampling design provided the same sample area to survey area ratio within and

among all locations and strata, i.e. ~8% of each location and 8% of each distance strata in 'concentrated' locations was sampled at each time. Earlier work indicated that juveniles would be well dispersed into the surrounding habitat within 8 days (Chapter 5, Experiment 5). However, this sampling design enabled distance strata to be equally proportionally sampled at each time and estimates of the mean total number of abalone to be made without relying on the assumption of their even distribution. This design also enabled all locations to be sampled within one day using a three person dive team at each sampling time. Sampling at each location at 769 days post-release was within solid habitat, due to the substantial disturbance of boulder habitat within about 4 months of sampling (inferred from clean rock surfaces and lack of algal growth on disturbed boulders), and utilised 1 m^2 quadrats haphazardly cast within each location (n = 5; Table 6.1; Section 6.2.4.2 Sampling strategy).

The mean total number of abalone at each 'concentrated' location, prior to 769 days post-release, was calculated as the sum of the total number of abalone within each of the distance strata. The standard error for the total number of abalone within each location was calculated as the sum of the standard errors from samples taken within each distance stratum multiplied by the proportion of the total area occupied by each stratum. Abalone collected during sampling at 363 and 769 days post-release were measured for growth (Table 6.1; Section 6.2.4.3 Growth).

Experiment 3 - replicated and controlled release of juveniles

Experiment three was designed to measure the impact of releasing juvenile abalone on 1) the total number of abalone surviving; 2) the total number of all wild abalone; and 3) the number of wild abalone in four length classes (very-small (0<30 mm); small (30<60 mm); medium (60<115 mm); large (≥115 mm)), within a naturally depleted wild population, through time for >2 years. It was hypothesised that the total number of all abalone (released and wild) at release locations would be significantly greater than at control locations and this would be consistent through time, for >2 years. It was also hypothesised that the total number of all wild abalone and wild abalone in each of the four length classes would not be significantly different at release or control locations through time, for >2 years. Finally, as locations were within relatively close proximity and there was no differentiation between abalone released among locations, it was hypothesised that there would be no significant difference in the mean length (mm) of abalone released among release locations after >2 years. It was predicted that juveniles released to locations would survive and grow at similar rates through time, their number would significantly enhance those of wild stocks and that the release of abalone would not have a negative impact on the number of wild conspecifics.

Four release and control locations were identified along a 5 km length of coast immediately south of Kiama, NSW, Australia (Locations A-D and E-H, respectively, Figure 6.1). The

abundance of wild *H. rubra* within locations was not quantitatively assessed prior to the release of abalone. Qualitative assessment during surveys to define location boundaries identified low abundances (<10 emergent individuals) of extant populations of *H. rubra* within all locations (personal observation). At each of the four release locations (A-D) 9 000 abalone (14 mm SL) were released in eleven devices, each holding approximately 820 abalone.

Sampling at the release and control locations 108 and 280 days post-release consisted of disturbed boulder sampling within quadrats haphazardly distributed throughout each location (n = 10; Table 6.1; Section 6.2.4.2 Sampling strategy). Sampling at 777 days post-release was within solid habitat only (n = 5; Table 6.1; Section 6.2.4.2 Sampling strategy). Sampling within solid habitat at this time was done for reasons described in Experiment 2 i.e. substantial disturbance of boulder habitat within about 4 months of sampling. At each time the number of released abalone and the number of wild abalone in the four length classes was counted. The length class of wild abalone was determined by comparison of the shell length with known lengths marked on the diver's slate. At 108 days post-release, up to the first ~50 individuals observed were collected and measured for growth from each location. All abalone observed at subsequent sampling times were collected and measured for growth (Table 6.1; Section 6.2.4.3 Growth).

6.2.2.2. Single locations

A number of batches of abalone were released at single locations. The release of abalone in experiments 4-6 was done with the objective to obtain estimates of the variation in survival and growth of released abalone through time at locations separated by large distances. It was predicted that the total numbers of juveniles would decline through time and that rates of survival would decline such that they became similar among locations after long periods of time (>1 year).

Experiment 4 - Fingal Island - South, Port Stephens

One location (1 025 m^2) was defined on the south side of Fingal Island, Port Stephens, NSW (Figure 6.1). At this location 80 000 abalone (7 mm SL) were released in 43 devices (Table 6.1). Short-term sampling (<60 days post-release) was done at four times (1, 9, 23 and 57 days post-release) with samples from boulder collections or boulder sampling *in situ*, taken from within 3 m of five devices, together with the number of abalone remaining on devices counted at each time (n = 5; Table 6.1). Sampling after 60 days was by boulder sampling with disturbance within haphazardly cast 1 m^2 quadrats (n = 10; Table 6.1). The total number of juveniles within the location at <60 days post-release was calculated as the mean number of abalone.m^{-2} within a 3 m radius of each of the five devices, multiplied by the number of devices released to the location (n = 43). Sampling at times >60 days post-release consisted of disturbed

boulder sampling within quadrats haphazardly distributed throughout each location (n = 10; Table 6.1; Section 6.2.4.2 Sampling strategy). The number of abalone sampled and removed from the location during sampling at 1 and 9 days were subtracted from the total number released to calculate the percent of abalone surviving after 60 days. All abalone collected at 407 days post-release were measured for growth (Table 6.1; Section 6.2.4.3 Growth). No abalone were observed at 794 days post-release.

Experiment 5 - Red Point, Eden

One location (136 m^2) was defined approximately 500 m south of Red Point, Eden, NSW (Figure 6.1). At this location 32 000 abalone (7 mm SL) were released in 20 devices (Table 6.1). These abalone were released using CCA-devices as this batch of juveniles was among the first produced from the hatchery and methods for their release were not fully tested. The effect of rough seas on the survival of released abalone was advantageously observed within 10 days after the release of these abalone (Table 6.1). Sampling at 154 and 463 days post-release was by boulder sampling with disturbance within haphazardly cast 1 m^2 quadrats (n = 10 and 5, respectively). Sampling at 916 days post-release was by the same method within 0.36 m^2 quadrats (n = 10; Table 6.1; Section 6.2.4.2 Sampling strategy). Levels of replication varied through time as resources for long-term sampling changed. All abalone collected at 463 and 916 days post-release were measured for growth (Table 6.1; Section 6.2.4.3 Growth).

Experiment 6 - Steam Trawler Bay, Eden

One location (240 m^2) was defined approximately 500 m south of Mowarry Point, Eden, NSW (Figure 6.1). At this location 25 500 abalone (14 mm SL) were released in 32 devices. Sampling was done 93, 274 and 455 days post-release (Table 6.1). Sampling at each time was done by boulder sampling with disturbance within haphazardly cast 0.36 m^2 quadrats (n = 15, 10 and 15, respectively; Table 6.1; Section 6.2.4.2 Sampling strategy). Levels of replication varied through time as resources for long-term sampling changed. All abalone collected at 455 days post-release were measured for growth (Table 6.1; Section 6.2.4.3 Growth).

6.2.3. Bio-economic assessment of a stock enhancement program

A deterministic model was constructed to investigate the economic returns of a simulated abalone release program, using parameters described in Table 6.3. The model considered the net present value (NPV) per year, for ten years, of investing in the release of 100 abalone per year for the first 5 years of a program. The release of a nominal 100 abalone was chosen as the inputs and results could be simply scaled up via a linear scale. A discount rate of 10% p.a. was used as an alternate investment opportunity to reflect the uncertainty in returns from the enhancement program (Caddy & Defeo 2003). Abalone were theoretically released at two lengths, 7 and

15 mm. These lengths were used to reflect those released in experiments 2-6 (this study) and represent lengths at which natural mortality rates decline rapidly from ~2.5 (Shepherd & Breen 1992), whilst also providing a contrast in production costs. The cost of producing abalone was calculated at \$AUD 0.02 mm^{-1}, with the understanding that economies of scale for these production costs would not be generated from the production of 100 abalone each year but likely 3-4 orders of magnitude more (Hart *et al.* 2007). The length of legal size abalone was set at the NSW state-wide minimum legal length of 117 mm (as at July 2008). Mean weight of legal size abalone was 0.259 kg (Andrew *et al.* 1996). The beach price (\$AUD kg^{-1} paid to shareholders in the NSW commercial abalone fishery by processors) was \$AUD 34.kg^{-1} (D. Worthington, Abalone Council of NSW, personal communication, 2009) and within \$AUD 2 kg^{-1} of that paid between 2003-04 (Anon 2005c). Beach prices of \$AUD 24 and \$AUD 44.kg^{-1} were used to provide pessimistic and optimistic contrast in potential financial returns, respectively. Growth rates used were an average from locations displaying relatively slow (G_I) and fast (G_{III}) growth rates from Experiment 3 and Experiment 2 (this study; Table 6.5), respectively, and were combined with an average of the two highest growth rates described for tagged and recaptured *H. rubra* in NSW in Worthington *et al.* (1995) (G_{II}). The average of the highest two growth rates from Worthington *et al.* (1995) was used, given that a release program targeting economic return is likely to favour areas of faster growth rather than areas of reef that demonstrate less productive growth characteristics, indicated by Worthington *et al.* (1995). Survival regimes included instantaneous rates of natural mortality (M) adapted from Shepherd and Breen (1992), results of survival after 2 years (4%; Experiment 2, this study; Table 6.3) and survival after 2 years corrected for sampling efficiencies (x1.5 to 6%; Table 6.3; after Shepherd 1998, Guzman del Proo *et al.* 2004, Dixon *et al.* 2006).

The model progressed in annual time steps applying investment inputs (expenses) at the beginning of each year, i.e. the cost of releasing 100 abalone at \$AUD 0.02 mm^{-1} for the first 5 years. Rates of growth and mortality were applied at each time step as outlined for each scenario (Table 6.3). The proportion of legal size abalone surviving at a given year were harvested. The total number of abalone harvested each year was multiplied by the mean weight of a legal size abalone and the beach price, to generate an annual income. The expenses and income for each year were summed to generate an annual cash flow. The NPV of the initial investment and annual cash flows were calculated for each year, from the second year of the program, at a discount rate of 10% (Caddy & Defeo 2003, Roberts *et al.* 2007b). For example, in scenario 5, for 7 mm abalone, one hundred abalone were released in the first year, and at each subsequent year for the next 4 years at an investment input cost of \$AUD 14 yr^{-1}. These abalone grew at 35.1 $mm.yr^{-1}$ for the first two years after release, and 23.4 $mm.yr^{-1}$ for the third year to reach legal size in the fourth year. As the abalone reached at or above legal size in the fourth

year, the proportion of those surviving in that year were harvested. The total number of abalone available for harvest in each year was multiplied by the mean weight and beach price. This provided an income for each year that abalone survived to legal size, i.e. for years 5-9 of the program. Given these expenses and income statistics the annual cash flow of the release program was used to calculate the NPV each year over the term of the program (10 years).

6.2.4. General protocols

6.2.4.1. Location selection

Each location consisted of an area of reef physically dominated by boulder field habitat with smaller areas of solid habitat (see List of Terms; Table 6.1) with a primary cover of crustose coralline alga and other encrusting alga and stands of brown foliose alga, commonly either *Ecklonia radiata* or *Phyllospora comosa*. Areas dominated by articulated coralline alga and other turfing alga, or containing large quantities of sediment among boulders and habitat less likely to support abalone, including Barrens (Underwood *et al.* 1991, Andrew & Underwood 1992), were avoided. Locations known to have supported commercially fished populations, that had subsequently declined, and locations known to be heavily fished and productive (NSW abalone industry members, personal communication) were preferred. To minimise emigration, all release locations were selected with some natural boundaries of inappropriate or less preferred abalone habitat (e.g. sand, Barrens or inter-tidal areas).

6.2.4.2. Sampling strategy

A general sampling strategy was determined for the long-term temporal sampling of release locations. Standard sampling times to obtain long-term estimates of survival and growth were maintained within windows of between 3-6, 9-15 and either ≥18 or ≥24 months, for released larvae and juveniles, respectively (Table 6.1). Estimates of density derived from sampling within boulder field habitat, using disturbed boulder sampling (BQ; Chapter 5, Section 5.2.5 Sampling techniques) were assumed to be representative of the whole release location. This assumption was made as solid habitat provided similar physical habitat attributes and abalone (released and wild) could be observed within the complex, concreted substratum commonly making up solid habitat within locations. The mean (+ SE) total number of abalone surviving within a location was calculated as the mean (+ SE) of the product of the density estimate from each replicate sample (abalone.m^{-2}) and the total area of the location (m^2; or the reduced total area, for samples taken within solid habitat; Table 6.1), unless otherwise stated in the text.

Sampling within solid habitat was done due to large-scale natural physical disturbance of boulder field habitat, by rough seas, and the inaccuracy of measures of density within locations derived from sampling within severely disturbed boulder field habitat (Chapter 5, Experiment

8). Where sampling within solid habitat was done the total area of each location was reduced to that of the solid habitat only. The area of solid habitat within a location was calculated as the total area of the location minus that of boulder field habitat. The area of boulder field habitat was calculated as the product of the total area of the location and the proportion of quadrats able to be sampled, using disturbed boulder sampling, from the total number required to be haphazardly cast to obtain the required sample size, determined from previous sampling times.

6.2.4.3. Growth

The total shell length (SL mm), and that of the green tagged shell in the spire of abalone recaptured after release as juveniles, was measured to the nearest 0.5 mm using vernier calipers. The green tagged shell was used to describe the length of each individual at release (see Chapter 3). All abalone collected during sampling were cleaned of any bio-fouling on the spire to enable the tagged green shell to be easily seen and measured. Rates of growth were derived from the difference in the length of the green tagged shell, the total shell length and the number of days post-release that individuals were recaptured (Table 6.1). All abalone observed during sampling after ~1 year were collected and measured except where otherwise stated in the text and with the exception of very few individuals that were damaged during collection or could not be collected or measured *in situ*.

6.2.4.4. Data analysis

Analysis of variance (ANOVA) was used to test for differences among treatments (Table 6.2). All analyses consisted of orthogonal designs. In all models, factors were analysed as fixed factors, with the exception of the factor Location in experiments 2 and 3, where it was analysed as a random factor. Cochran's *C*-test was used to test for homogeneity of variances and data were transformed as described in the text. Where data were heterogeneous after transformation ANOVA was still performed on transformed data, as the method is robust to heterogeneity of data, and results were conservatively interpreted with $P<0.01$ (Underwood 1997). Where repeated analyses were performed on the same series of data, i.e. Experiment 3, the significance levels of the tests were corrected for multiple comparisons using the standard Bonferroni adjustment. The use of ANOVA to test for significant differences in mean lengths of abalone among locations (Experiment 3) was done on unbalanced data (*n* ranged between 12 and 16; Table 6.5) at 777 days post-release.

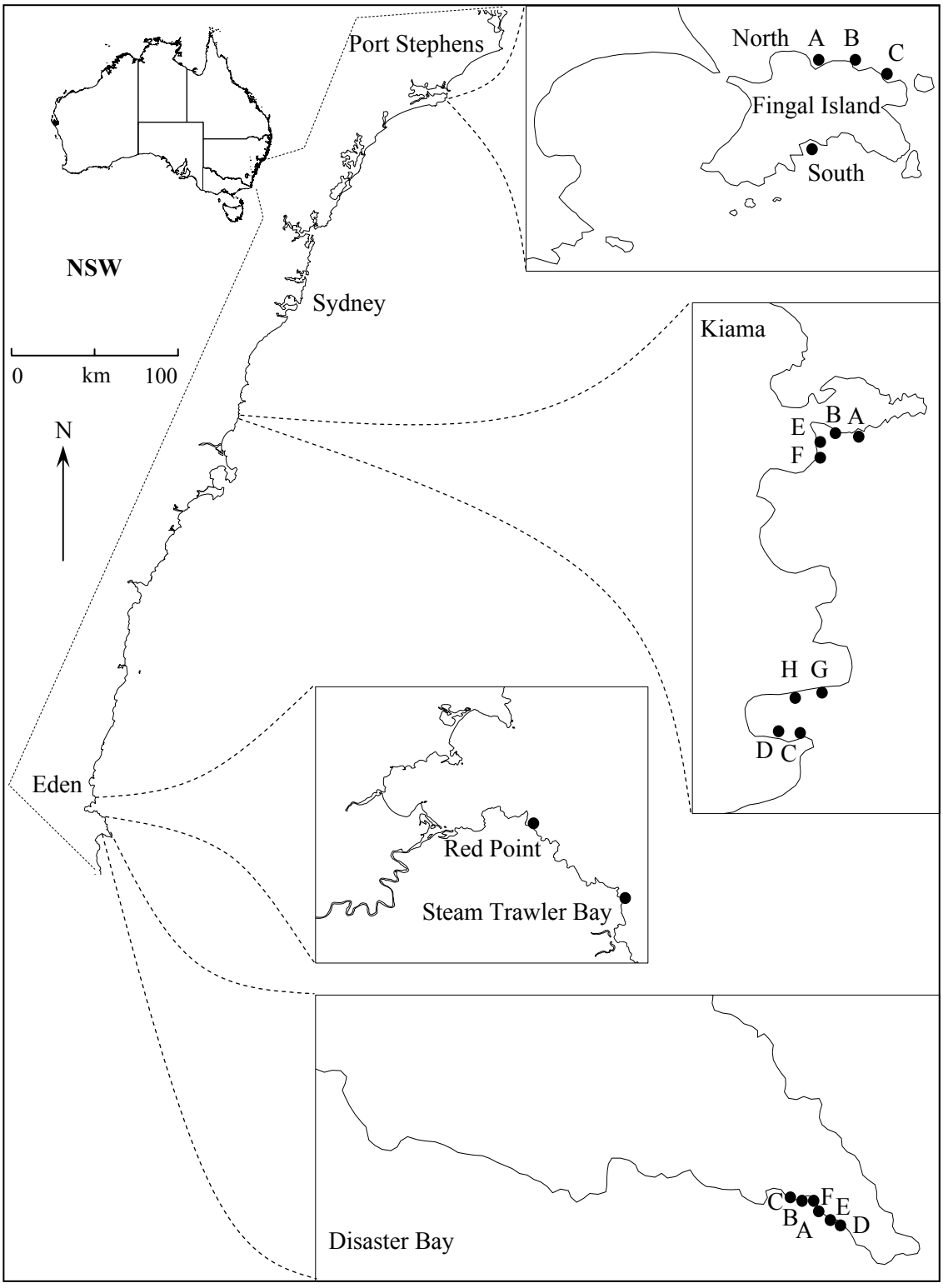


Figure 6.1 Map of Australia and the NSW coast showing survey locations. Insets are not to scale.

Table 6.1 Locations and an outline of release and sampling methods used at each location through time. Sampling methods include boulder collection (BC), boulder sampling *in situ* (B*is*), disturbed boulder sampling (BQ) and disturbed boulder samples in solid habitat (S) (for method details see Chapter 5). 'Reduced area' describes the re-defined total area of the release location after habitat loss for each sampling time.

Expt No.	Location name	Length at release (mm)	Number released (Lav. x 10^6) (Juv. x 10^3)	Habitat type (percentage of solid substrata)	Area of locations (m^2) (reduced area)	Days sampled post-release (and years)	Growth (mm) (tag & shell length) (see Table 6.5)	Sample method	Replicates (n)	Area sampled per replicate (m^2)
Larvae										
1	Fingal Is. North A	Lav.	1.10	boulder field & solid	375	1		BC	5	0.05 (boulder)
	Fingal Is. North B			(38%)		2		BC	5	0.05 (boulder)
	Fingal Is. North C					14		BC	5	0.05 (boulder)
						56 (0.2)		BC	5	0.05 (boulder)
						196 (0.5)	Yes	Bis	20	0.05 (boulder)
						412 (1.1)	(no abalone)	Bis	10	0.05 (boulder)
						553 (1.5)	Yes	BQ	5	1.00
Juveniles										
2	Disaster Bay A - diffuse	15	1.0	boulder field & solid	115	7		BQ	25	0.36
	Disaster Bay B - diffuse			(42%)		63 (0.2)		BQ	25	0.36
	Disaster Bay C - diffuse					183 (0.5)		BQ	25	0.36
						363 (1)	Yes	BQ	25	0.36
					(54)	769 (2.1)	Yes	S	5	1.00
	Disaster Bay D - concentrated	15	1.0	boulder field & solid	113	7		BQ	25	0.36
	Disaster Bay E - concentrated			(41%)		63 (0.2)		BQ	25	0.36
	Disaster Bay F - concentrated					183 (0.5)		BQ	25	0.36
						363 (1)	Yes	BQ	25	0.36
					(54)	769 (2.1)	Yes	S	5	1.00
3	Kiama A	14	9	boulder field & solid	97	108 (0.3)	Yes	BQ	10	0.36
	Kiama B			(34%)		280 (0.8)	Yes	BQ	10	0.36
	Kiama C				(34)	777 (2.1)	Yes	S	5	0.36
	Kiama D									
	Controls (Kiama E , F, G, H)		0	(46%)	89 (41)	as above				
4	Fingal Is - South	7	80.0	boulder field & solid	1025	1		BC	5	0.27 (boulders)
				(30%)		9		BC	5	0.27 (boulders)
						23		Bis	5	0.27 (boulders)
						57 (0.2)		Bis	5	0.27 (boulders)
						407 (1.1)	Yes	BQ	10	1.00
						794 (2.1)	(no abalone)	BQ	10	1.00
5	Red Point	7	32.0	boulder field & solid	136	20		Bis	5	0.24 (boulders)
				(22%)	(64)	154 (0.4)		BQ	10	1.00
					(64)	463 (1.3)	Yes	BQ	5	1.00
					(64)	916 (2.5)	Yes	BQ	10	0.36
6	Steam Trawler Bay	14	25.5	boulder field & solid	240	93 (0.3)		BQ	15	0.36
				(41%)		274 (0.8)		BQ	10	0.36
						455 (1.2)	Yes	BQ	15	0.36

Table 6.2 Design of analyses investigating the long-term (769 days) survival of juveniles after diffuse or concentrated release (Experiment 2) and the difference in i) total number of abalone (released and wild); ii) total number of all wild abalone; iii) very-small (<30 mm) wild abalone; iv) small (30<60 mm) wild abalone; and v) medium (60<115 mm) wild abalone at release and control locations through time (Experiment 3). Treatments for each factor are shown in brackets and described in the text.

Expt. No.	Factors [and treatments] in analyses	Length at release (mm)	Replicates (*n*)	Response variable
2	Release [Diffuse, Conc.]			
	Location [1, 2, 3]	15	5	Total number of released abalone
3	Time [1, 2, 3]			
	Treatment [Release, Control]			
	Location [1, 2, 3, 4]	14	5	Total number of all abalone (released and wild)
				Total number of all wild abalone
				Total number of wild abalone (<30 mm)
				Total number of wild abalone (30<60 mm)
				Total number of wild abalone (60<115 mm)
3	Location [1, 2, 3, 4]	14	12-16	Total length of released abalone

Table 6.3 Parameters used to determine net present value (NPV) of a program of releasing abalone for 5 years, calculating NPV each year for 10 years. Common parameters and scenarios describing different growth and survival regimes are shown as $G_{I\text{-}III}$ and $S_{I\text{-}III}$, respectively and described in the text. Annual growth rates at 65, 90 and 115 mm are shown as g_{65}, g_{90}, g_{115}, respectively. Growth rate for G_I g_{65} and G_{III} g_{65} and G_{III} g_{90} are from means among locations in Experiments 3 and 2 in this study, respectively. Other growth rate data are modified from Worthington *et al.* (1995) as described in the text.

	Investment (No. released / year for the first 5 years)	Discount rate (% p.a)	Length at release (mm)	Production cost ($AUD.mm^{-1})	Legal size (mm)	Mean weight legal size (kg)	Beach price (live abalone) ($AUD.kg^{-1})
Common parameters	100	10	7, 15	0.02	117	0.259	24, 34, 44

	Growth rate (mm.yr^{-1})				Survival	
		g_{65}	g_{90}	g_{115}		
Scenario 1	G_I	25.6	23.4	11.7	S_I	Linear decline in M from 2.5 to 0.3 for 3 years M = 0.2 for subsequent years
Scenario 2	G_I	25.6	23.4	11.7	S_{II}	4% survival at 2 years M = 0.2 for subsequent years
Scenario 3	G_I	25.6	23.4	11.7	S_{III}	6% survival at 2 years M = 0.2 for subsequent years
Scenario 4	G_{II}	35.1	23.4	11.7	S_I	Linear decline in M from 2.5 to 0.3 for 3 years M = 0.2 for subsequent years
Scenario 5	G_{II}	35.1	23.4	11.7	S_{II}	4% survival at 2 years M = 0.2 for subsequent years
Scenario 6	G_{II}	35.1	23.4	11.7	S_{III}	6% survival at 2 years M = 0.2 for subsequent years
Scenario 7	G_{III}	43.3	41.8	11.7	S_I	Linear decline in M from 2.5 to 0.3 for 3 years M = 0.2 for subsequent years
Scenario 8	G_{III}	43.3	41.8	11.7	S_{II}	4% survival at 2 years M = 0.2 for subsequent years
Scenario 9	G_{III}	43.3	41.8	11.7	S_{III}	6% survival at 2 years M = 0.2 for subsequent years

6.3. RESULTS

6.3.1. Long-term survival and growth of larvae

Experiment 1 - survival and growth of released larvae

The mean total number of abalone surviving within locations at Fingal Island (FI) - North A-C declined rapidly within 60 days post-release (Figure 6.2). Two days after release, the mean total number of settled larvae among locations was 33 450 (Table 6.4; range 30 600 - 38 250), indicating <5% of larvae successfully settled within each location. At 56 days post-release the mean total number of abalone at location FI - North B was 1 275 individuals. No abalone were observed at the other two locations at this time. From 56 days post-release no abalone were observed at location North A. The mean total number of abalone at locations North B and C was 637 and 1 500 individuals, respectively after 196 days post-release. No abalone were detected at any of the three locations after 412 days. At 553 days post-release the mean total number of abalone within a location was 125 individuals (range 0 - 300; Figure 6.2 and Table 6.4; note: all reference to abalone refers to positively identified, batch-tagged abalone only, unless otherwise stated). Variation among replicate samples was high, ranging between 25-100% of the mean, for samples taken after 56 days. All abalone sampled within 60 days post-release and six (from a total of seven) and five (from a total of nine) sampled at 196 and 553 days post-release, respectively, were positively identified from the calcein labelled larval shell visible in the spire of the recaptured individuals. The mean growth of abalone from settlement to 196 and 553 days was 32.6 and 27.2 $mm.yr^{-1}$, respectively (Table 6.5).

6.3.2. Long-term survival and growth of juveniles

6.3.2.1. Replicated locations

Experiment 2 - diffuse and concentrated release of juveniles

There was no significant difference in the mean percent survival of abalone released in a diffuse array compared with those released in a concentrated group at locations in Disaster Bay after 769 days. However, there was significant variation in the mean percent survival of abalone among locations (Figure 6.3 and Table 6.6). The mean percent survival of abalone among locations ranged between 0-9% at 769 days post-release. Variation among replicate samples was high, ranging between 51-100%.

The mean total number of abalone surviving among locations and within the diffuse and concentrated treatments at Disaster Bay ranged between 0-83 and 11-91 individuals, respectively, at 769 days post-release (Figure 6.4). The mean percent survival of abalone within

diffuse and concentrated treatment locations declined to between 10-20% and 7-38% within 183 days, respectively. At 363 days post-release the mean percent survival of abalone within diffuse treatment locations (A-C) was 12.8% compared with 3.8% within concentrated treatment locations (D-F; Table 6.5). However, there was substantial variation among locations within the diffuse treatment, with the mean percent survival within locations (A-C) ranging between 5-23% (Figure 6.4). High mean total numbers of abalone detected 6 days post-release at concentrated treatment locations (D-F) was a consequence of high numbers of abalone (up to 86) detected within replicates within 2 m of devices, and single replicates at 3-5 m or 5-6 m, where a small aggregation of between 2-3 individuals was detected.

Equal total numbers of abalone were recaptured within diffuse and concentrated treatment locations, respectively, at 769 days post-release (Table 6.5). There was a significant difference in the total length ($t_{(2)} = 2.37$, p=0.05, df = 18) and annual growth rate ($t_{(2)} = 2.96$, p=0.05, df = 18) of abalone among diffuse and concentrated treatments at 769 days post-release (Figure 6.5 and Table 6.5). Abalone recaptured within concentrated treatment locations had a mean shell length of 107.3 mm (range: 100-116 mm) compared to 99.1 mm (range: 84-112 mm) for those in diffuse treatment locations at 769 days post-release. The difference in mean length among treatments after 363 days was ~10 mm (63.1 mm and 54.0 mm at concentrated and diffuse treatment locations, respectively). The mean rate of growth of abalone released in a concentrated group (43.8 $mm.yr^{-1}$) was ~4 $mm.yr^{-1}$ greater than those released in a diffuse array (39.7 $mm.yr^{-1}$) after 769 days post-release (Table 6.5). The mean growth among all locations was 43.3 $mm.yr^{-1}$ and 41.8 $mm.yr^{-1}$, after 363 and 769 days post-release, respectively (as used in growth scenario G_{III} in the bio-economic assessment; Table 6.3).

Experiment 3 - replicated and controlled release of juveniles

Long-term total abundance within release and control locations

There were significantly greater numbers of all abalone (released and wild) among release locations at Kiama compared with control locations, where abalone had not been released, and this pattern was consistent among sampling times (Figure 6.6 and Table 6.7). There was little difference in the mean total number of all abalone among release locations within sampling times, with the exception of Location C at 108 days post-release. The mean total number of all abalone among release locations was 1 825 (range 200-2800), 1 423 (range 1080-1875) and 528 (range 329-628) at consecutive sampling times, respectively (Figure 6.6, dashed lines). Similarly, the mean total number of all abalone among control locations within sampling times was consistent, averaging 49 (range 45-55), 175 (range 135-275) and 178 (range 150-200) individuals at consecutive sampling times, respectively (Figure 6.6, dashed lines). Released abalone comprised between 88 - 99%, 75 - 88%, and 42 - 58% of the total abalone population at

each time, respectively. No released abalone were found at any control locations. A reduction in total area of 65% (97 m^2 to 34 m^2) and 54% (89 m^2 to 41 m^2) for release and control locations, respectively, at 777 days post-release (Table 6.1), coincided with an increase in mean density of small and medium wild abalone among all locations (see below for wild abalone). This increase in density, despite the reduction in total area, contributed to similar total numbers of wild abalone within control locations between 280 and 777 days post-release. The mean percent of wild abalone at release locations increased from 17% (range 12-25%) at 280 days post-release to 50% (range 42-58%) at 777 days post-release (Figure 6.6, shaded bars within release locations). At Location C at 108 days post-release there was a mean total of 200 individuals detected, an order of magnitude fewer individuals than at the other three release locations and ~1 600 less than detected at this location at 280 days post-release. Large north-easterly swell conditions at 108 days post-release, poor visibility and reduced daylight during sampling likely contributed to this estimate.

Long-term abundance of wild populations at release and control locations

There was no significant effect of releasing abalone on the mean total number of all, very-small, small and medium size wild abalone among locations at Kiama through time (Figure 6.7 and Table 6.8). No large abalone were observed within locations at any sampling time. The mean total number of all wild abalone increased significantly through time and this was consistent among treatments (release and control) and locations (Figure 6.7 and Table 6.8). At 108 days post-release the mean total number of all wild abalone among locations was 47 (range 25-70) individuals (Figure 6.7, dashed lines). There was little difference in the mean total number of all wild abalone among locations between 280 days (203 individuals; range 135-292) and 777 days post-release (222 individuals; range 137-343; Figure 6.7).

There was a significant effect of time on the mean total number of medium size wild abalone (Table 6.8). However, this analysis was done on transformed data displaying heterogeneous variances and as such this result is interpreted with caution (Underwood 1997). The mean total number of medium size wild abalone increased among locations at successive sampling times from 10 (range 0-55) to 52 (range 0-110) and 125 (range 69-247) individuals, respectively (Figure 6.7). The increase in abundance of medium size wild abalone from 280 to 777 days post-release was the consequence of an increase in mean density of medium size wild abalone within release (0.2 to 4.3 m^{-2}) and control (0.6 to 3.2 m^{-2}) locations, respectively. This increase in density was coincident with, or related to, a mean reduction in total area of release and control locations of 65% (97 to 34 m^2) and 54% (89 to 41 m^2), respectively (Table 6.1).

There was a pulse of very-small wild abalone detected among locations at 280 days post-release where the mean total number of this length class was 68 (range 0-125; Figure 6.7, dashed line).

Similarly, the mean total number of small wild abalone increased between 108 days (30; range 0-50) and 280 days (125; range 45-240) post-release (Figure 6.7, dashed lines). Together with the pulse increase in very-small and small length classes at 280 days post-release, the increase in mean total number of medium size wild abalone between 280 and 777 days post-release indicated the progression of a cohort from a natural recruitment event likely to have occurred prior to the release of abalone. From the almost complete lack of detection of very-small wild abalone at 108 days post-release it is likely that individuals from this recruitment pulse were too small to detect underwater at this time. However, it seems that this event subsequently contributed numbers of wild abalone to the very-small (<30 mm) and small (30-60 mm) length classes, detected at 280 days post-release. In turn these individuals grew through to the medium (60-115 mm) length class at 777 days post-release (Figure 6.7). Variation among replicate samples was high among treatments through time for each size class and ranged between 42-100%, 61-100%, 61-100% and 45-100% of the mean within all, very-small, small and medium size classes, respectively (Figure 6.7).

Long-term survival of released abalone

The mean total number of abalone (released abalone only) surviving among release locations at Kiama 777 days after the release of 9 000 juvenile abalone to each location, was 260 (range 192-357; Table 6.4 and Figure 6.8). The mean percent survival among locations declined steadily through time and was similar among locations within sampling times, ranging ≤10%, with the exception of that estimated at Location C 108 days post-release (Figure 6.8). Sampling at 108 and 280 days post-release provided estimates of between 2-31% and 9-18% abalone surviving within locations, respectively (Figure 6.8). At 777 days post-release the mean percent of abalone surviving among locations was 2.6% (range 2.1-4.0%; Table 6.4 and Figure 6.8).

Long-term growth of released abalone

There was a significant difference in the mean length of abalone among locations at Kiama, 777 days post-release (Figure 6.9 and Tables 6.5 and 6.9). At 777 days post-release the largest difference in mean length of abalone among locations was ~17 mm, between Locations A (77.3 mm) and B (60.1 mm), that were separated by ~300 m (Table 6.5 and Figure 6.1). The mean rate of growth of abalone recaptured at 777 days post-release was 25.6 mm.yr^{-1} (range 22.9-30.1 mm.yr^{-1}, Table 6.5; as used in growth scenario G_I in the bio-economic assessment, Table 6.3). Differences in the mean length of recaptured abalone varied among locations and increased through time, although minimum and maximum mean lengths at each time differed among locations, with the exception of the maximum mean length at Kiama A, from 280 days post-release (Table 6.5).

6.3.2.2. Single locations

Experiment 4 - Fingal Island - South, Port Stephens

Sampling within 60 days of the release of abalone at Fingal Island - South revealed a decline in the abundance of abalone to ~37 000 (46%; Figure 6.10 and Table 6.4). At 407 days post-release the total number of abalone within the location was ~2 500 (3.2%). Sampling at 794 days post-release did not detect any abalone. The mean length of abalone collected and measured at 407 days post-release was 32.0 mm, representing a mean growth rate of 22.7 $mm.yr^{-1}$ (Table 6.5).

Experiment 5 - Red Point, Eden

Sampling within 30 days of the release of abalone at Red Point revealed a decline in the abundance of abalone to ~5 500 (17.5%; Figure 6.11 and Table 6.4). Rough seas and associated habitat disturbance affected the survival of juveniles within 10 days post-release, with a small number of juveniles crushed between small boulders (personal observation). Sampling at 154 days post-release provided an estimate of ~1 800 (6%) abalone remaining. After 463 and 916 days post-release the total number of abalone surviving within the location was 205 (1.4%) and 59 (0.4%), respectively (Figure 6.11 and Table 6.4). The mean length of abalone recaptured at 463 and 916 days post-release was 46.2 and 82.9 mm, representing mean growth rates of 28.5 and 29.7 $mm.yr^{-1}$, respectively (Table 6.5). At each time the mean rate of growth varied among individuals by up to 19.4 and 11.2 $mm.yr^{-1}$, respectively.

Experiment 6 - Steam Trawler Bay, Eden

Sampling at 93 days post-release at Steam Trawler Bay revealed a decline in the abundance of abalone to ~11 600 (46%; Figure 6.12 and Table 6.4). Sampling at 274 and 455 days post-release provided estimates of ~1 700 (7%) and 267 (1%) abalone surviving, respectively. The mean length of abalone recaptured at 455 days post-release was 34.3 mm, representing a mean growth rate of 17.8 $mm.yr^{-1}$ (Table 6.5).

6.3.3. Bio-economic assessment of a stock enhancement program

Sustained negative rates of NPV were generated for all scenarios within the initial years where all released abalone were at lengths below the minimum legal length (MLL) and hence no income from their harvest was generated (Figure 6.13). Rates of NPV turned positive where annual income, from harvesting released abalone that had grown to legal length, exceeded annual investment (releasing abalone). Where release scenarios were assessed against the slowest growth rates (G_I), rates of NPV were negative throughout the release phase (first 5 years) as released individuals took 5 years to reach the MLL and hence no financial returns were received until the sixth year. Changes in annual rates of NPV varied among scenarios where

growth regimes (G_{II} and G_{III}) interacted with the different survival regimes (S_I, S_{II}, S_{III}). Increasingly positive cumulative cash flows and increasing NPVs resulted where growth and survival rates increased through time, and greater proportions of released individuals survived to reach the MLL and were harvested (Figure 6.13 and Table 6.3). Given the parameters described for the model, no positive NPVs could be gained from the release of 7 or 15 mm SL abalone given rates of survival described in survival regime I (S_I ; Figure 6.13; Scenarios 1, 4 and 7).

Positive NPVs of investing in a long-term (10 year) abalone release program were achieved with a beach price of \$AUD 34.kg^{-1} where the combination of rates of survival and growth in scenario 8, or where survival was described in S_{III}, irrespective of growth rates, with the release of 7 mm abalone (Figure 6.13; Scenarios 3, 6 and 9). There were positive financial returns where 7 mm juveniles were subject to rates of survival described for S_{III}, at a beach price of \$AUD 34.kg^{-1}. Moreover, increasing growth rates (G_I, G_{II} and G_{III}) resulted in released abalone reaching the minimum legal length (MLL) after 5, 4 and 3 years, respectively (Figure 6.13; Scenarios 3, 6 and 9). The reduced time to reach the MLL resulted in positive annual cash flows at the same respective times after the release of abalone and faster positive cumulative cash flows resulting in subsequently higher positive NPVs through time. NPV increased through time until all surviving abalone released in the fifth year were harvested. Once the surviving proportion of abalone that had been released in the fifth year had reached the MLL and been harvested, NPVs plateaued (Figure 6.13; Scenarios 6 and 9). This occurred as cumulative cash flow stopped increasing as there were no additional abalone growing to the MLL. Positive NPVs were gained within 9, 8 and 6 years where the beach price set at \$AUD 34.kg^{-1} and 7 mm SL abalone were released and subject to survival rates described in S_{III} and growth rates described in G_I, G_{II} and G_{III}, respectively (Figure 6.13; Scenarios 3, 6 and 9). The more optimistic beach price of \$AUD 44.kg^{-1} provided positive investment scenarios for all 7 mm SL abalone released and subject to survival regimes S_{II} and S_{III}, irrespective of growth rates. However, NPVs became positive within shorter periods of time as growth rate increased and annual cash flow increased with increasing survival (Figure 6.13; Scenarios 2, 5, 8 and 3, 6, 9).

Investing in the release of 15 mm SL abalone provided positive NPVs where the beach price was set at the more optimistic \$AUD 44.kg^{-1}, survival rates were as described for S_{III} and where the two faster growth rates (G_{II} and G_{III}) were used (Figure 6.13; Scenarios 6 and 9). In both of these cases released abalone reached the MLL at 3 years. This provided positive rates of NPV from that year (although the NPV was still negative). These positive rates of NPV continued even while investment continued with the release of abalone in years 4 and 5. The maximum positive rate of NPV occurred as investment stopped in year 5 and abalone continued to be harvested as they reached the MLL, up to year 8. NPV plateaued from year 8 as all abalone released in year 5 grew to a length ≥MLL and were harvested in this year (Figure 6.14; Scenarios 6 and 9).

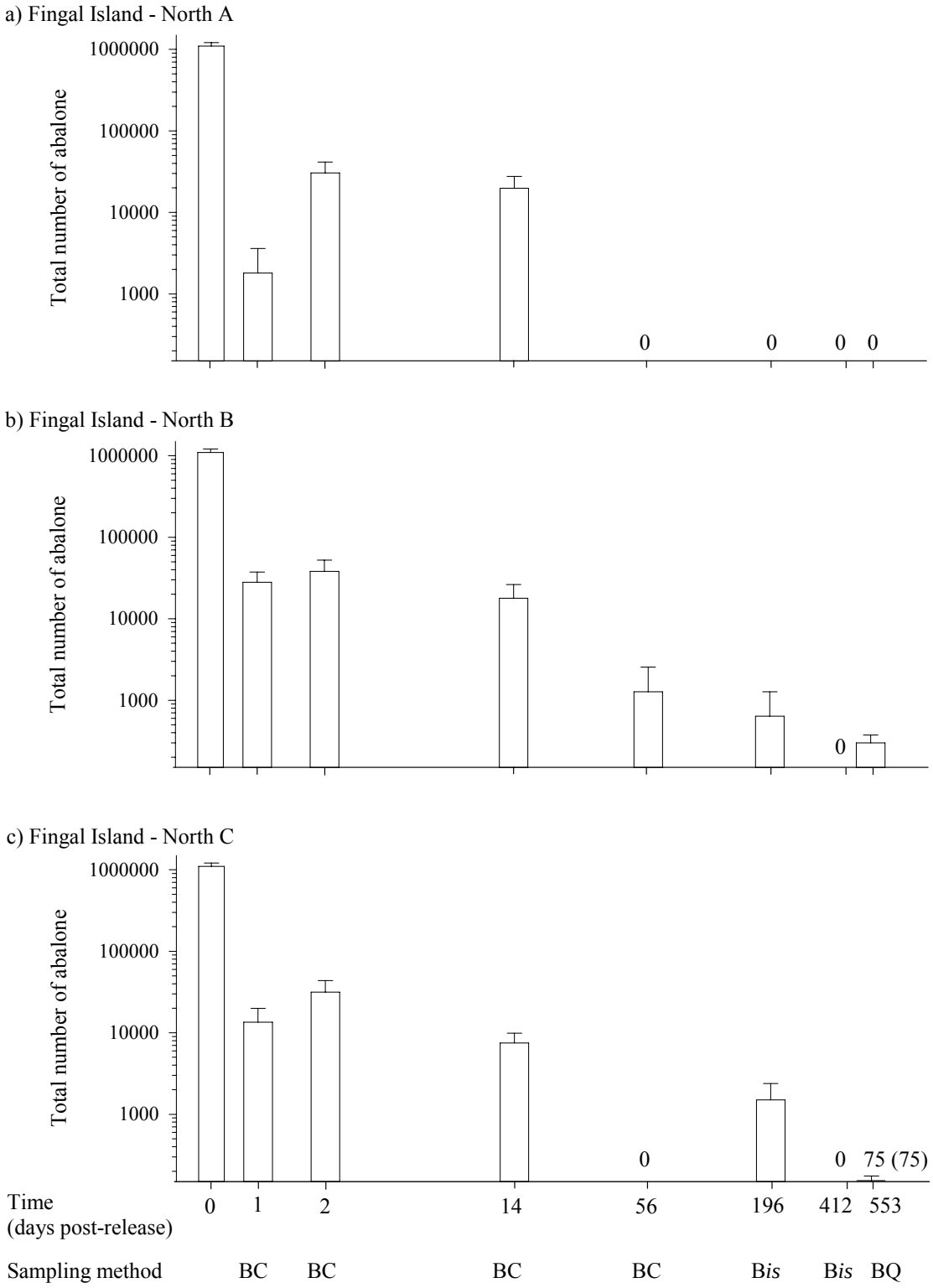


Figure 6.2 Experiment 1. Mean total number of larvae released (+ SE) and number of abalone at Fingal Island - North A - C, at release (Time 0) and through time (days post-release). Samples were collected using boulder collections (BC), boulder sampling *in situ* (B*is*) and disturbed boulder quadrats (BQ) within 1 m^2 (n = 5, 20, 10 and 5 at days 1-56, 196, 412 and 553, respectively). Note, these data represent batch-tagged abalone only.

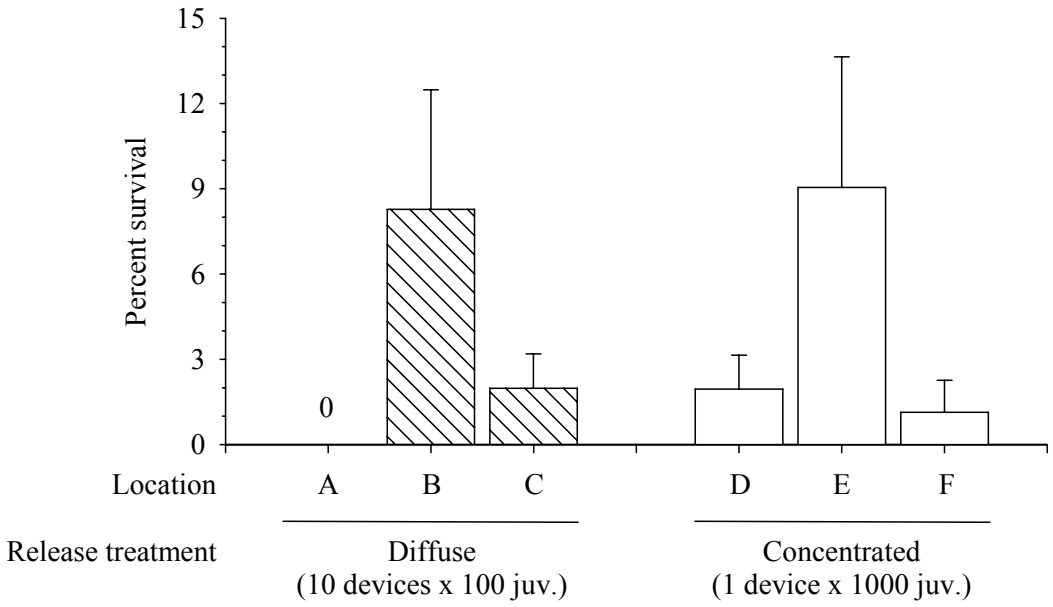


Figure 6.3 Experiment 2. Mean percent survival of abalone (+ SE) at Disaster Bay release locations, using diffuse (Locations A - C) and concentrated (Locations D - F) release of juveniles, 769 days after release. Sampling was by disturbed boulder sampling in solid habitat within 1 m^2 quadrats (n = 5). Note, these data represent batch-tagged abalone only.

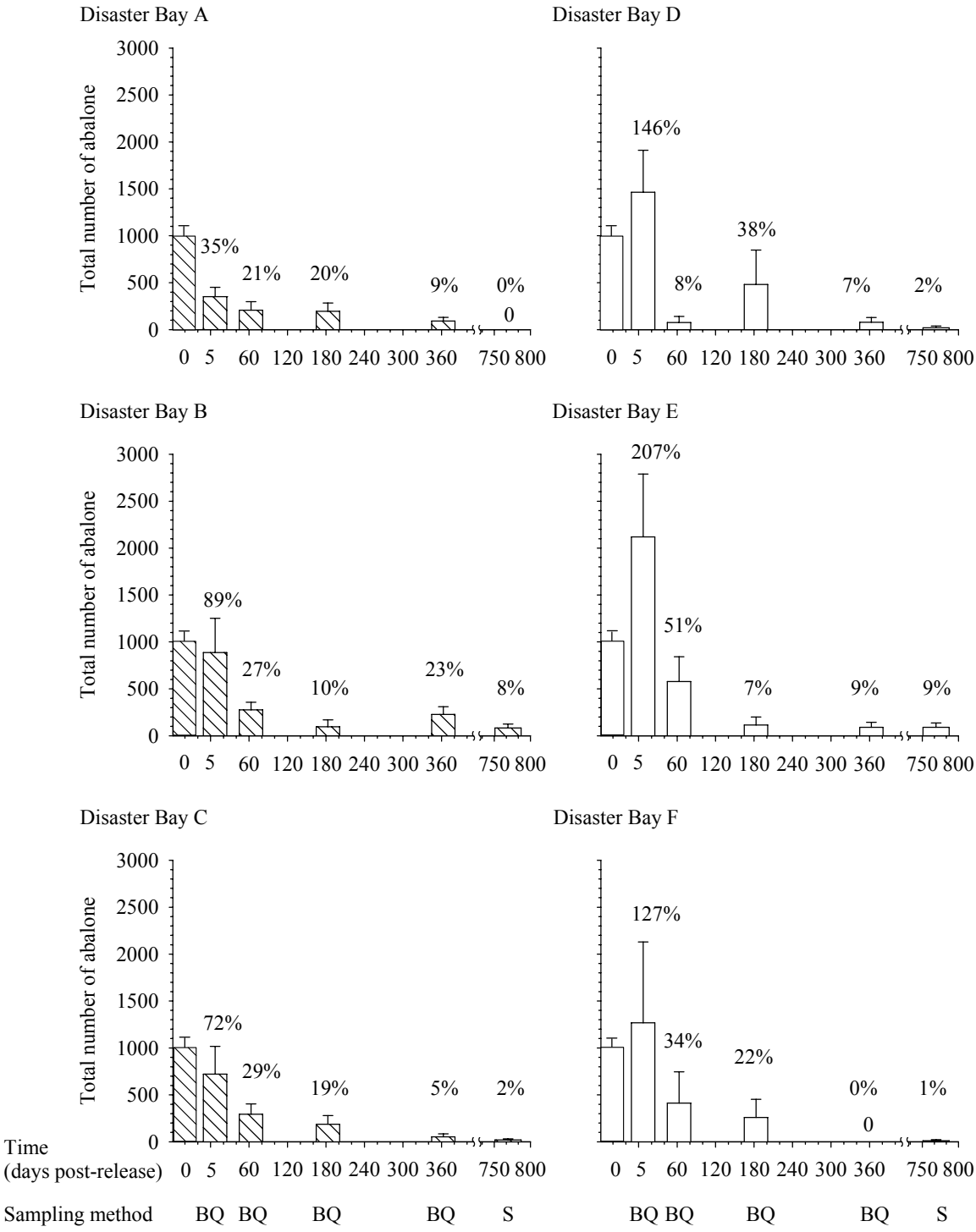


Figure 6.4 Experiment 2. Mean total number of abalone (+ SE) at Disaster Bay release locations (A - F) at release (Time 0) and through time (days post-release). Juveniles were released at locations A - C using a diffuse release method (hashed bars; 10 devices, each holding 100 juveniles, evenly placed throughout each location), and at locations D - F using a concentrated release (open bars; one device holding 1 000 juveniles, placed at the centre of each location). Sampling up to 363 days was done by disturbed boulder quadrats (BQ) within 0.36 m^2 (n = 25). Sampling at 769 days was by disturbed boulder sampling in solid habitat (S) within 1 m^2 quadrats (n = 5). Numbers indicate mean percent survival at each time. Note, these data represent batch-tagged abalone only.

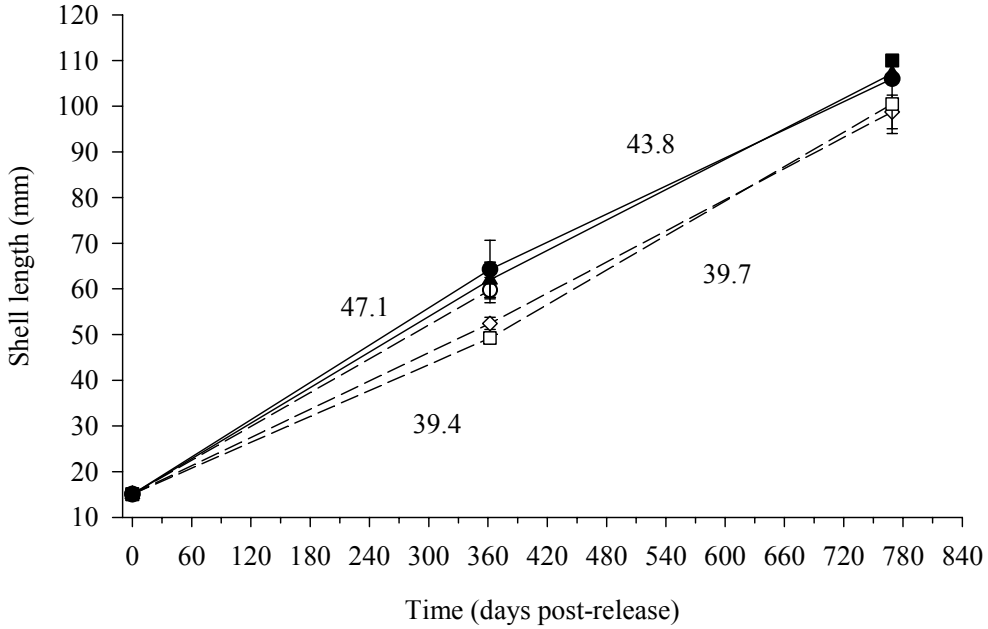


Figure 6.5 Experiment 2. Mean length (mm) (± SE) of abalone recaptured at Disaster Bay release locations, 363 and 769 days post-release (diffuse: -○- A; -◊- B; -□- C and concentrated: -●- D; -▲- E; -■- F; *n* varies among locations through time, see Table 6.5). Numbers below and above lines indicate mean growth rates ($mm.yr^{-1}$) at diffuse and concentrated release locations, respectively. Note, these data represent batch-tagged abalone only.

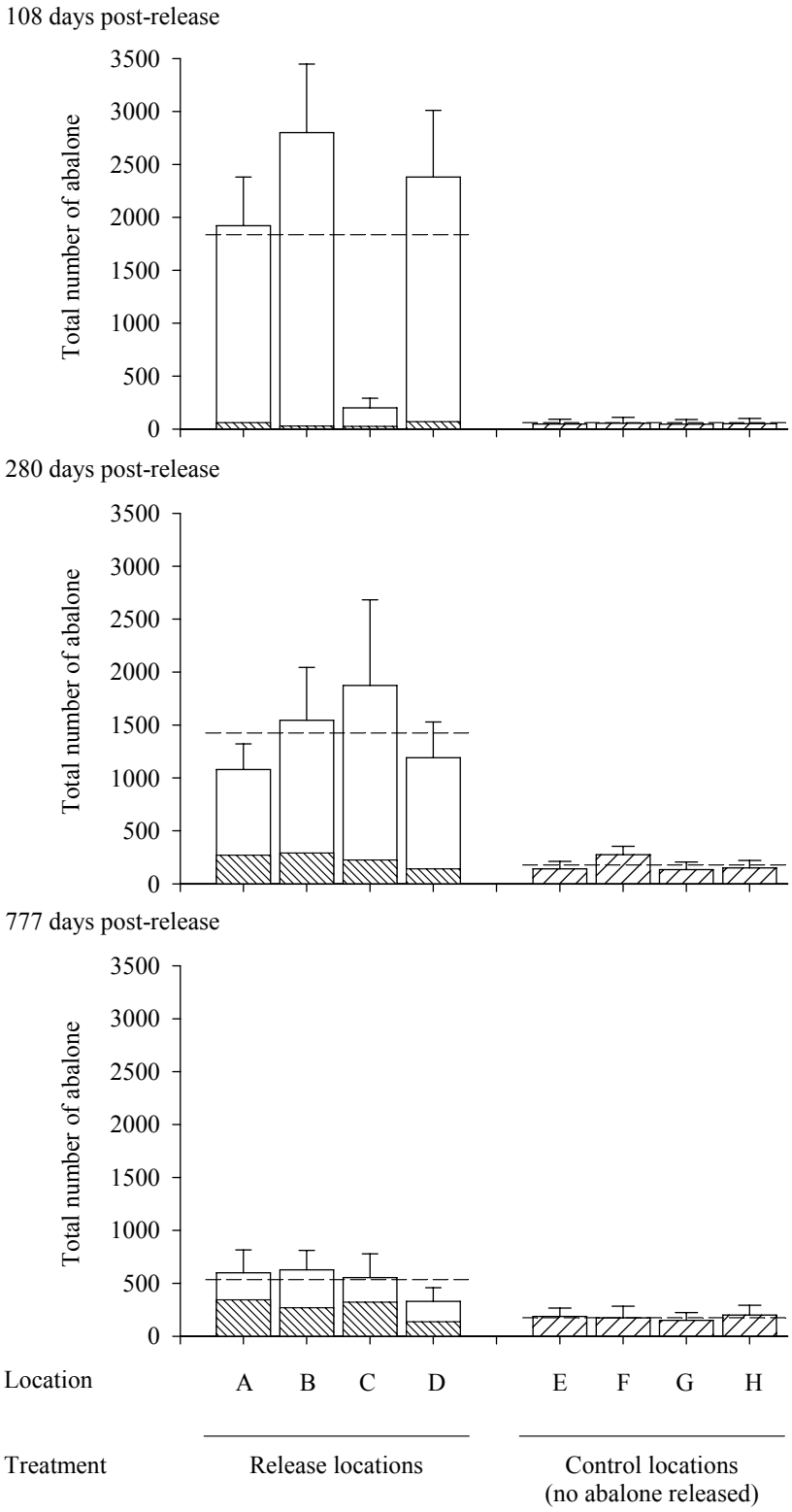


Figure 6.6 Experiment 3. Mean total number (+ SE) of all abalone (batch-tagged; open bars and wild; hashed bars) at four release (A - D) and four control (E - H) locations at Kiama. Sampling was done within 0.36 m^2 quadrats by disturbed boulder sampling at 108 and 280 days post-release (n = 10 and 5 for release and control locations, respectively) and disturbed boulder sampling in solid habitat at 777 days after release (n = 5). Shading within release locations represents the mean total number of wild abalone. Dashed lines indicate mean total numbers of all abalone among locations within each treatment.

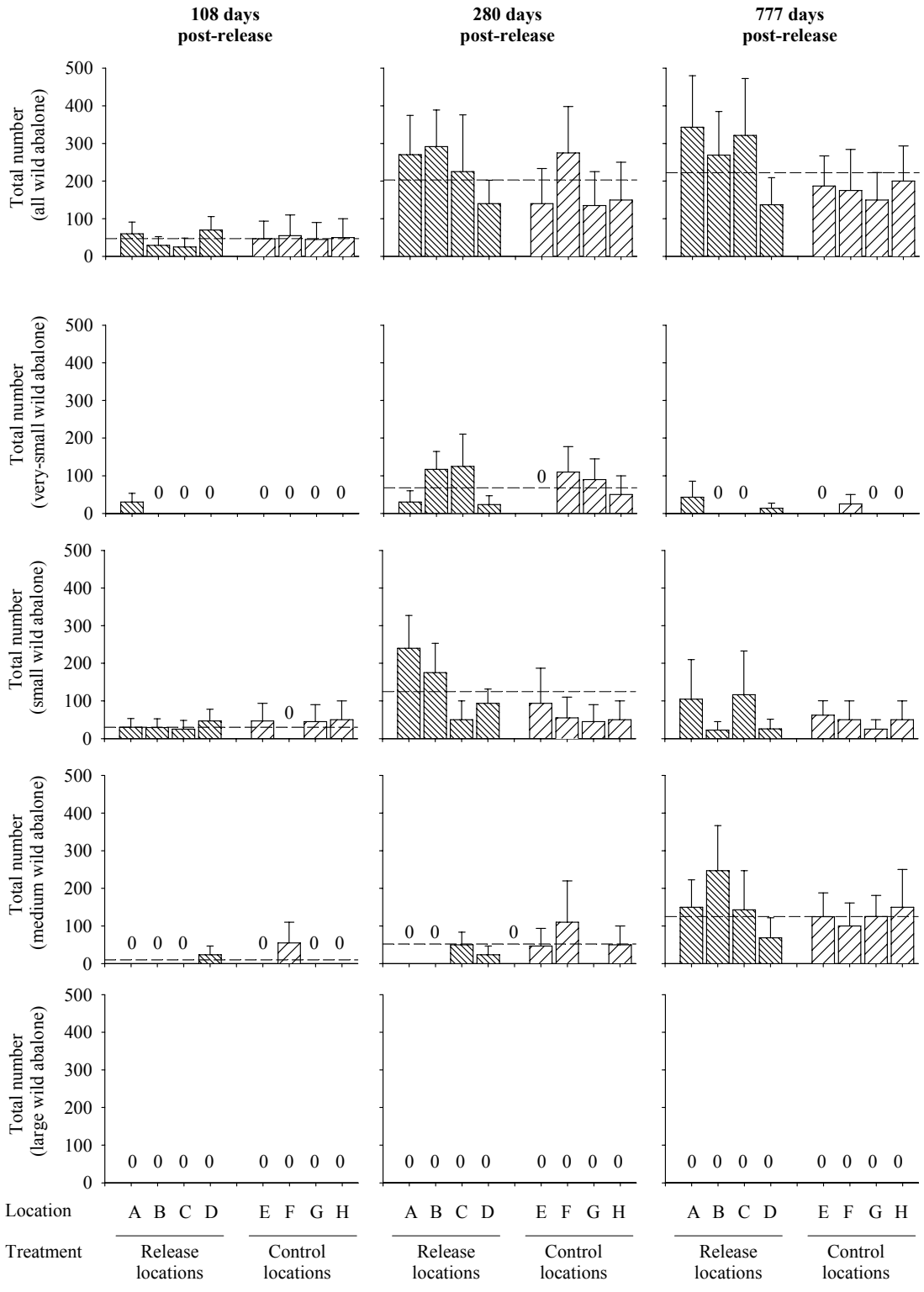


Figure 6.7 Experiment 3. Mean total number (+ SE) of all, very-small (<30 mm), small (30<60 mm), medium (60<115 mm) and large (≥115 mm) wild abalone at four release (A - D; dense hashed bars) and control (E - H; light hashed bars) locations at Kiama, 108, 280 and 777 days after the release of juveniles to locations A - D (all released abalone are excluded from these data). Sampling was done by disturbed boulder sampling (BQ) at 108 and 280 days after release (n = 10 and 5 for release and control locations, respectively) and disturbed boulder sampling in solid habitat at 777 days after release (n = 5), within 0.36 m^2 quadrats. Dashed lines indicate mean numbers of abalone among locations.

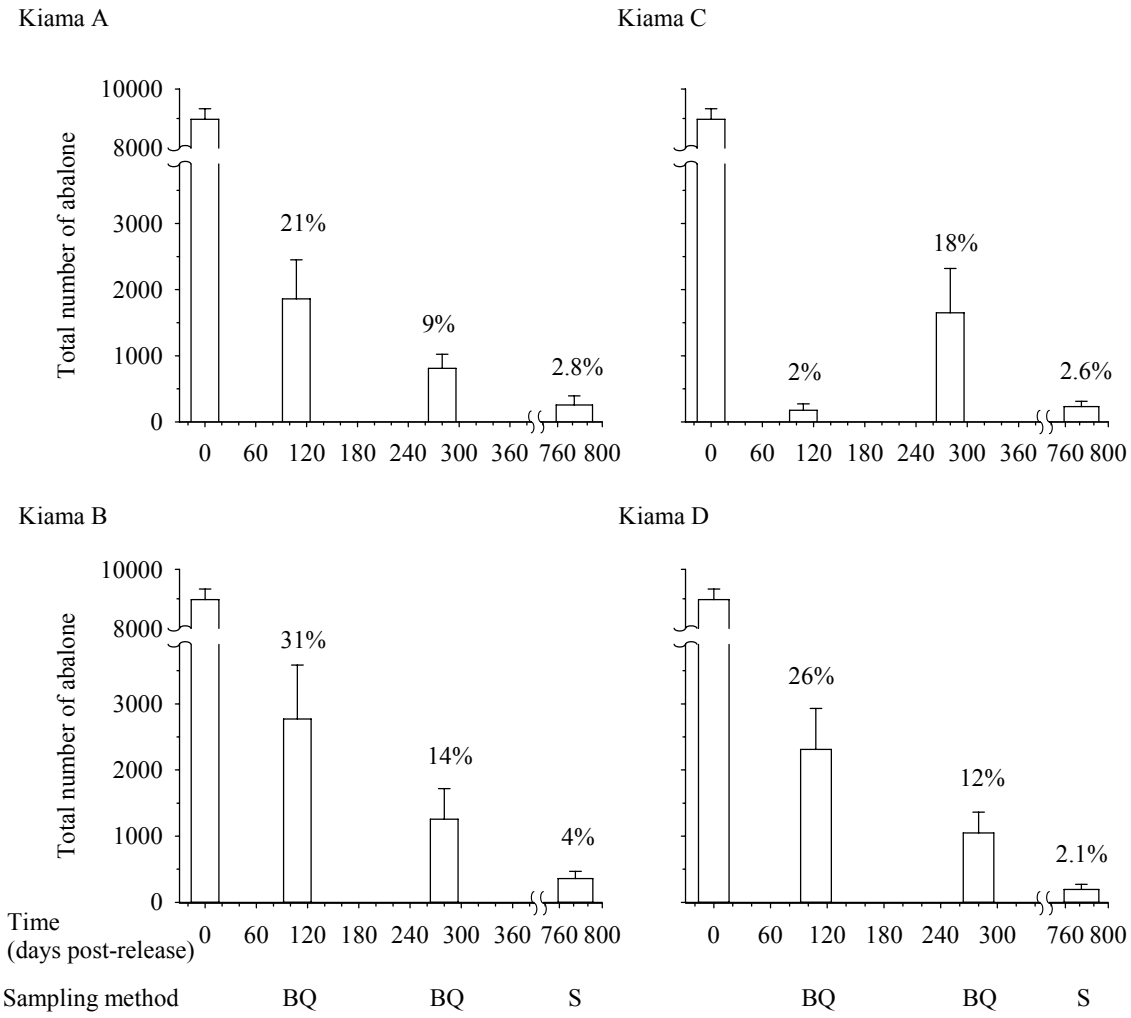


Figure 6.8 Experiment 3. Total number of abalone (+ SE) at four release locations at Kiama at release (Time 0) and through time (days post-release). Sampling was done by disturbed boulder sampling (BQ) and disturbed boulder sampling in solid habitat (S) within 0.36 m^2 quadrats (n = 10 and 5, respectively). Numbers indicate mean percent survival at each time. Note, these data represent recaptured batch-tagged abalone only.

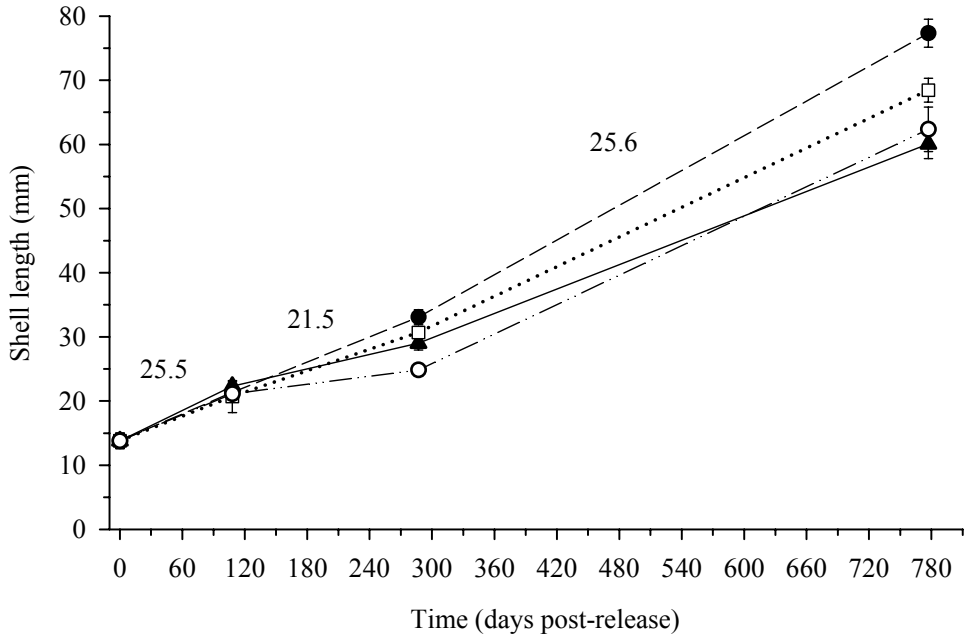


Figure 6.9 Experiment 3. Mean length (mm) (± SE) of abalone recaptured at Kiama release locations after each sampling time (-●- A; -▲- B; -□- C; -○- D; *n* varies among locations through time, see Table 6.5). Numbers above lines indicate mean growth rates ($mm.yr^{-1}$). Note, these data represent batch-tagged abalone only.

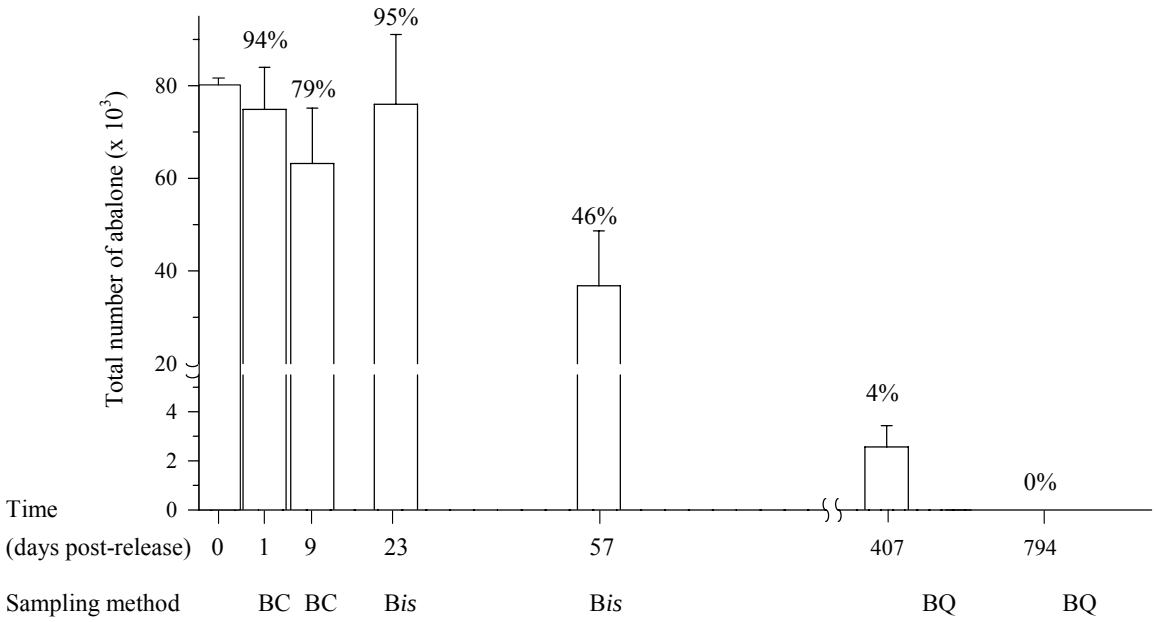


Figure 6.10 Experiment 4. Mean total number of abalone (+ SE) at Fingal Island - South through time. Sampling was done using boulder collections (BC) (n = 6 at 1 and 9 days after release), boulder sampling *in situ* (B*is*) (n = 6 at 23 and 57 days post-release) and disturbed boulder sampling (BQ) within 1 m^2 (n = 10 at 407 and 794 days after release). Numbers indicate mean percent survival at each time. Note, these data represent batch-tagged abalone only.

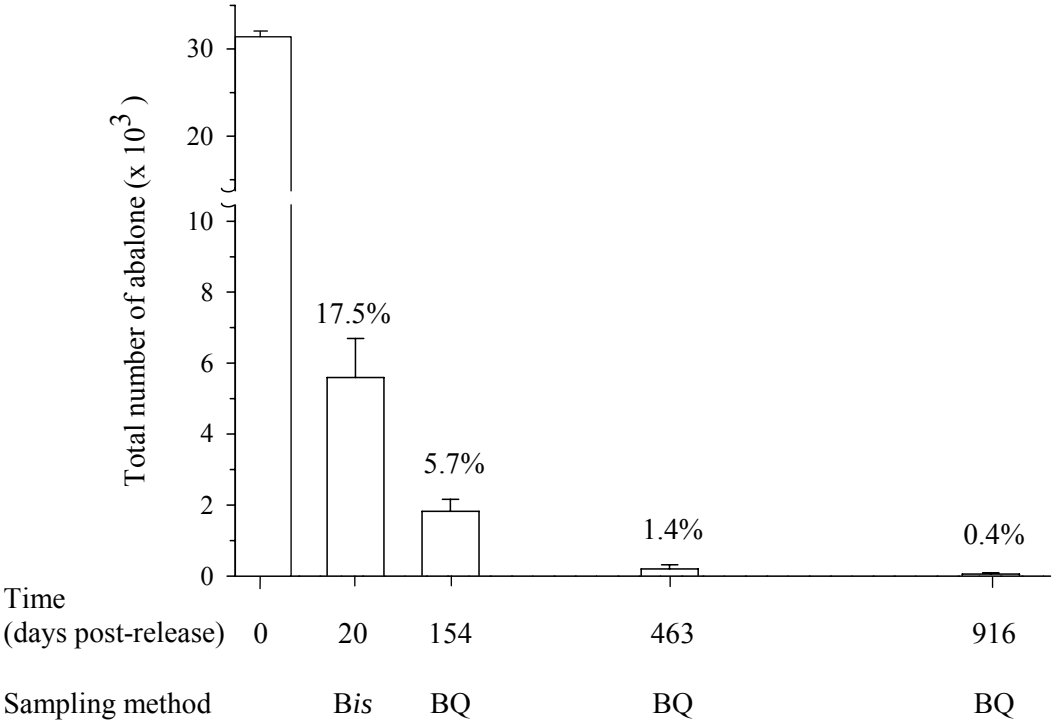


Figure 6.11 Experiment 5. Mean total number of abalone (+ SE) at Red Point through time. Sampling was done using boulder sampling *in situ* (B*is*) (n = 6 at 20 days after release) and disturbed boulder sampling (BQ) within 1 m^2 (n = 10 and 5 at 154 and 463 days after release, respectively) and within 0.36 m^2 (n = 10 at 916 days post-release). Numbers indicate mean percent survival at each time. Note, these data represent batch-tagged abalone only.

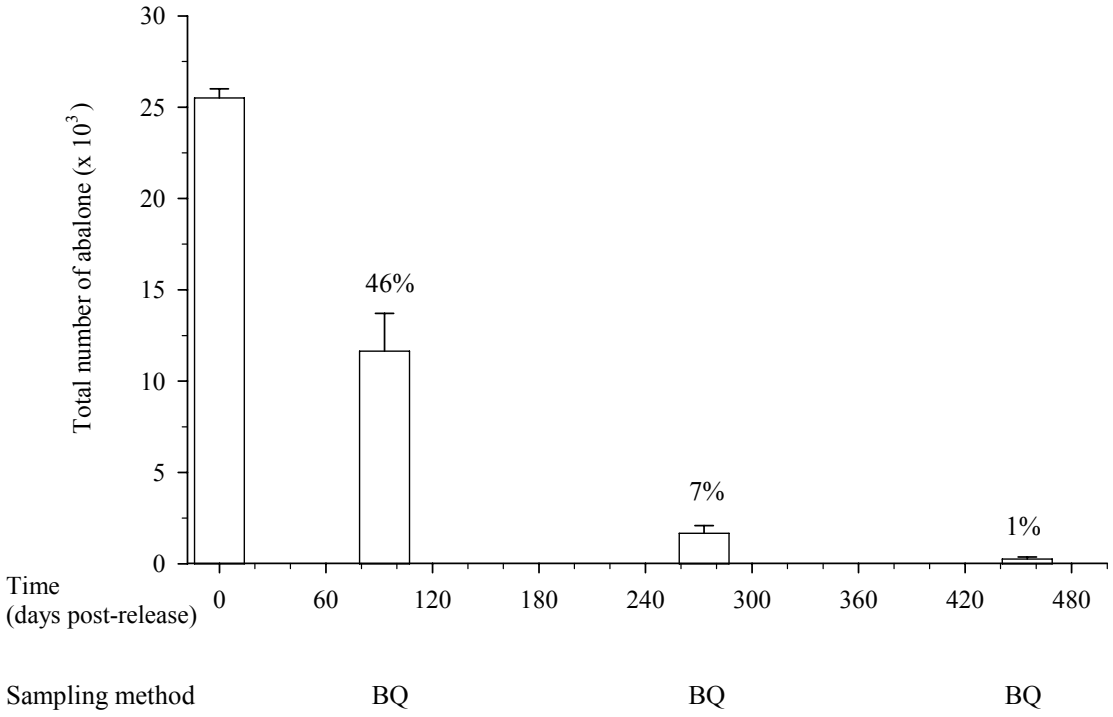


Figure 6.12 Experiment 6. Mean total number of abalone (+ SE) at Steam Trawler Bay through time. Sampling was done using disturbed boulder sampling (BQ) within 0.36 m^2 (n = 15, 10 and 15 at 93, 274 and 455 days post-release, respectively). Numbers indicate mean percent survival at each time. Note, these data represent batch-tagged abalone only.

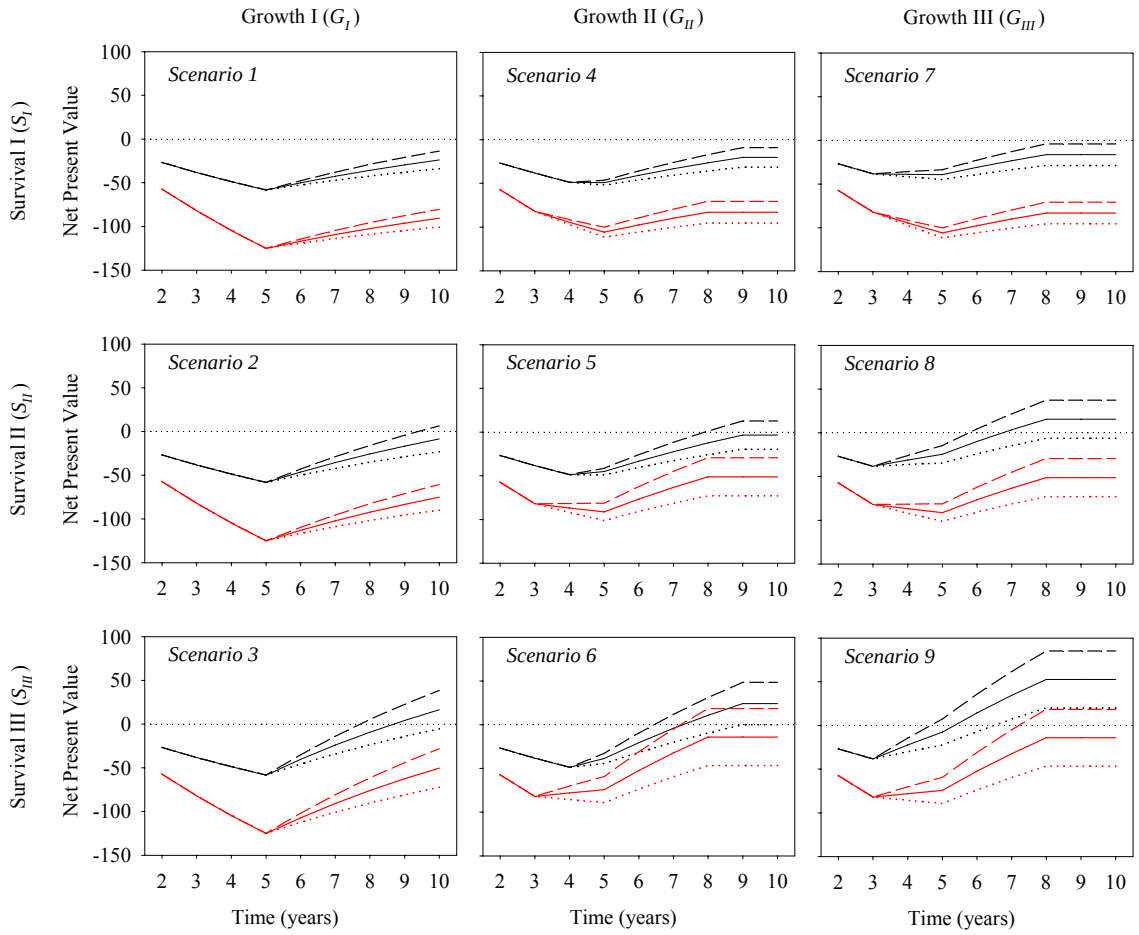


Figure 6.13 Net Present Value of releasing 100 abalone.year^{-1} for 5 years at a length of 7 mm (black lines) and 15 mm (red lines) at beach prices of 24 (····), 34 (—) and 44 (---) \$AUD kg^{-1} at a discount rate of 10%, given scenarios described in the text and outlined in table 6.3. The dotted line indicates a neutral investment strategy, 'break even point', against a relative investment returning 10% p.a. Results above and below the dotted line indicate a more or less profitable investment strategy compared with an alternative returning 10% p.a., respectively.

Table 6.4 The number released and number.m^{-2}, total number, survival (percent) and mortality (M.yr^{-1}) of released abalone through time, shown as the average from among locations at each time (total number and survival (percent) of abalone at individual locations through time is presented on respective figures and described in the text). 'Reduced area' indicates the altered total area of the location at respective times after release, due to a reduction in the physical habitat at locations as a result of rough seas.

Expt No.	Location name	Length at release (mm)	Number released (Lav. x 10^6) (Juv. x 10^3)	Area of locations (m^2) (reduced area)	Days sampled post-release (and years)	Number.m^{-2}	Total number	Survival (%)	Instantaneous mortality (M.yr^{-1})
Larvae									
1	Fingal Island - North A	Lav.	1.10	375	1	38.53	14450	1.31	1581.3
	Fingal Island - North B				2	89.20	33450	3.04	637.5
	Fingal Island - North C				14	40.13	15050	1.37	111.9
					56 (0.2)	1.13	425	0.04	51.2
					196 (0.5)	1.90	713	0.06	13.7
					412 (1.1)	0.00	0	0.00	-
					553 (1.5)	0.33	125	0.01	6.0
Juveniles									
2	Disaster Bay A - difuse	15	1.0	115	7	5.74	660	66.0	21.7
	Disaster Bay B - difuse				63 (0.2)	2.26	260	26.0	7.8
	Disaster Bay C - difuse				183 (0.5)	1.37	158	15.8	3.7
					363 (1)	1.11	128	12.8	2.1
				(54)	769 (2.1)	0.67	36	3.6	1.6
	Disaster Bay D - concentrated	15	1.0	113	7	15.26	1726	172.6	-28.5
	Disaster Bay E - concentrated				63 (0.2)	2.67	302	30.2	6.9
	Disaster Bay F - concentrated				183 (0.5)	1.96	222	22.2	3.0
					363 (1)	0.33	38	3.8	3.3
				(54)	769 (2.1)	0.73	39	3.9	1.5
3	Kiama A	14	9	97	108 (0.3)	18.26	1772	19.7	5.5
	Kiama B				280 (0.8)	12.57	1219	13.5	2.6
	Kiama C			(34)	777 (2.1)	7.64	260	2.9	1.7
	Kiama D								
4	Fingal Island - South	7	80.0	1025	1	73.10	74893	93.6	24.1
					9	61.68	63187	79.0	9.6
					23	74.19	76006	95.0	0.8
					57 (0.2)	35.93	36806	46.0	5.0
					407 (1.1)	2.50	2561	3.2	3.1
					794 (2.1)	0.00	0	0.0	-
5	Red Point	7.3	32.0	136	20	41.14	5595	17.5	32
					154 (0.4)	13.42	1825	5.7	7
				(64)	463 (1.3)	3.20	205	0.6	4
				(64)	916 (2.5)	0.93	59	0.2	3
6	Steam Trawler Bay	14	25.5	240	93 (0.3)	48.52	11644	45.7	3.1
					274 (0.8)	6.94	1667	6.5	3.6
					455 (1.2)	1.11	267	1.0	3.7

Table 6.5 Mean shell length (mm) (± SE) of the tagged shell (from the spire of recaptured abalone), total shell length, growth rate and number of batch-tagged, recaptured abalone measured at individual locations and pooled among locations (Totals), at sampling days post-release.

Expt No.	Location name	Tagged shell length (mm)			Total shell length (mm)			Growth rate (mm.yr^{-1})			Number of individuals measured per time		
Larvae	*Days post-release*	*196*	*412*	*553*	*196*	*412*	*553*	*196*	*412*	*553*	*196*	*412*	*553*
1	Fingal Island - North A	larvae	larvae	larvae	-	-	-	-	-	-	0	0	0
	Fingal Island - North B	larvae	larvae	larvae	18.0 ± 2.0	-	41.8 ± 5.5	33.5 ± 3.7	-	27.6 ± 3.6	2	0	4
	Fingal Island - North C	larvae	larvae	larvae	17.3 ± 0.3	-	39.0	32.1 ± 0.5	-	25.7	4	0	1
	Total (all locations pooled)				**17.5 ± 0.6**	**-**	**41.2 ± 4.3**	**32.6 ± 1.1**	**-**	**27.2 ± 2.8**	**6**	**0**	**5**
Juveniles													
	Days post-release	*363*	*769*		*363*	*769*		*363*	*769*		*363*	*769*	
2	Disaster Bay A - difuse	15.1 ± 0.5	-		59.8 ± 2.8	-		45.0 ± 2.4	-		8	0	
	Disaster Bay B - difuse	15.1 ± 0.6	15.1 ± 0.8		52.4 ± 1.3	98.8 ± 3.7		37.7 ± 1.2	39.7 ± 1.5		18	8	
	Disaster Bay C - difuse	14.0 ± 1.1	17.0 ± 2.0		49.3 ± 1.3	100.5 ± 6.5		35.5 ± 0.8	39.6 ± 2.6		4	2	
	Total (locations pooled)	**14.9 ± 0.4**	**15.5 ± 0.7**		**54.0 ± 1.3**	**99.1 ± 3.1**		**39.4 ± 1.1**	**39.7 ± 1.2**		**30**	**10**	
	Disaster Bay D - concentrated	16.8 ± 0.9	16.0		64.3 ± 6.4	106.0		47.9 ± 5.9	42.7		4	1	
	Disaster Bay E - concentrated	16.0 ± 0.7	15.0 ± 0.8		62.0 ± 3.9	107.1 ± 2.0		46.4 ± 3.5	43.7 ± 0.8		4	8	
	Disaster Bay F - concentrated	-	14.0		-	110.0		-	45.60		0	1	
	Total (locations pooled)	**16.4 ± 0.6**	**15.0 ± 0.6**		**63.1 ± 3.5**	**107.3 ± 1.6**		**47.1 ± 3.2**	**43.8 ± 0.7**		**8**	**10**	
	Days post-release	*108*	*280*	*777*	*108*	*280*	*777*	*108*	*280*	*777*	*108*	*280*	*777*
3	Kiama A	13.0 ± 0.5	12.5 ± 0.6	13.3 ± 0.9	21.3 ± 0.8	33.1 ± 1.2	77.3 ± 2.2	28.2 ± 1.3	26.2 ± 1.2	30.1 ± 0.9	63	30	12
	Kiama B	14.8 ± 0.5	12.4 ± 0.6	11.3 ± 0.6	22.3 ± 0.7	29.0 ± 1.1	60.1 ± 2.3	25.2 ± 1.6	21.2 ± 1.0	22.9 ± 0.9	60	45	16
	Kiama C	11.6 ± 1.0	12.4 ± 0.5	10.5 ± 0.6	20.7 ± 2.5	30.7 ± 0.8	68.5 ± 1.9	30.8 ± 6.0	23.3 ± 0.8	27.2 ± 0.7	5	55	13
	Kiama D	14.6 ± 0.5	12.6 ± 0.6	12.6 ± 0.9	21.1 ± 0.7	24.8 ± 0.9	62.4 ± 3.5	22.1 ± 1.5	15.6 ± 0.7	23.4 ± 1.3	53	40	14
	Total (all locations pooled)	**14.0 ± 0.3**	**12.4 ± 0.3**	**11.9 ± 0.4**	**21.6 ± 0.4**	**29.3 ± 0.5**	**66.4 ± 1.5**	**25.5 ± 0.8**	**21.5 ± 0.5**	**25.6 ± 0.6**	**181**	**171**	**55**
	Days post-release	*407*	*794*		*407*	*794*		*407*	*794*		*407*	*794*	
4	Fingal Island - South	6.6 ± 0.3	-		32.0 ± 0.7	-		22.7 ± 0.5	-		20	0	
	Days post-release	*463*	*916*		*463*	*916*		*463*	*916*		*463*	*916*	
5	Red Point	10.0 ± 0.5	8.4 ± 0.3		46.2 ± 2.0	82.9 ± 4.6		28.5 ± 1.6	29.7 ± 1.8		16	5	
	Days post-release	*455*			*455*			*455*			*455*		
6	Steam Trawler Bay	12.2 ± 0.7			34.3 ± 1.2			17.8 ± 0.8			6		

Table 6.6 Experiment 2. Summary of analysis of variance in the percent survival of abalone at locations at Disaster Bay using the diffuse (Locations A - C) and concentrated (Locations D - F) release methods, 769 days post-release. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's $C = 0.3726^{\ddagger}$, ns; variances 6, df 4). $^{\ddagger}\sin^{-1}\sqrt{x}$ transformed. Note, these data represent batch-tagged abalone only.

	Source	df	MS	*F*	F vs
1	Release method, M	1	0.003	0.26 ns	3
2	Location, L	2	0.098	4.08 *	4
3	M x L	2	0.011	0.45 ns	4
4	Error	24	0.024		
5	Total	29			

Table 6.7 Experiment 3. Summary of analysis of variance in the number of all abalone (released and wild) through time at release and control locations at Kiama. The number of sample replicates at release locations at 108 and 280 days was reduced to 5, by random allocation, to enable a balanced design. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.004 (**), P<0.022 (*) and not significant (ns). Probability values of the F statistic were Bonferroni adjusted to account for 5 multiple comparisons. (Cochran's $C = 0.1307^{\dagger}$, ns; variances 24, df 4). †sqrt (x + 0.1) transformed.

	Source	df	MS	F	F vs
1	Time (T)	2	1.743	2.577 ns	5
2	Treatment (Tm)	1	29.005	224.180 **	6
3	T x Tm	2	0.446	0.574 ns	7
4	Location (L)	3	0.327	0.548 ns	8
5	T x L	6	0.676	1.133 ns	8
6	Tm x L	3	0.129	0.217 ns	8
7	T x Tm x L	6	0.777	1.301 ns	8
8	Error	96	0.597		
9	Total	119			

Table 6.8 Experiment 3. Summary of analysis of variance in the number of all wild abalone; wild abalone (<30 mm); wild abalone (30<60 mm) and; wild abalone (60<115 mm) through time at release and control locations at Kiama. The number of sample replicates at release locations at 108 and 280 days was reduced to 5, by their random allocation, to enable a balanced design. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.004 (**), P<0.022 (*) and not significant (ns). Probability values of the F statistic were Bonferroni adjusted to account for 5 multiple comparisons. (Cochran's *C*: all wild abalone (ns†); wild abalone (<30 mm)†*; wild abalone (30<60 mm)†*; and wild abalone (60<115 mm)†*; variances 24, df 4). †sqrt (x + 0.1) transformed.

		Wild abalone† all sizes		Wild abalone† Very-small (<30 mm)		Wild abalone† Small (30<60 mm)		Wild abalone† Medium (60≤115 mm)		F vs
Source	df	MS	F	MS	F	MS	F	MS	F	
1 Time (T)	2	6.334	45.036 **	0.258	3.484	0.640	3.587	5.122	161.720 **	5
2 Treatment (Tm)	1	0.713	8.414	0.020	0.259	0.803	35.462	0.007	0.205	6
3 T x Tm	2	0.370	2.968	0.014	0.649	0.096	0.409	0.161	1.433	7
4 Location (L)	3	0.257	0.654	0.027	0.420	0.248	1.171	0.079	0.359	8
5 T x L	6	0.141	0.358	0.074	1.161	0.178	0.842	0.032	0.144	8
6 Tm x L	3	0.085	0.216	0.076	1.184	0.023	0.107	0.034	0.153	8
7 T x Tm x L	6	0.125	0.317	0.022	0.346	0.235	1.108	0.112	0.512	8
8 Error	96	0.393		0.064		0.212		0.219		
9 Total	119									

Table 6.9 Experiment 3. Summary of analysis of variance in the mean length of abalone at release locations (A - D) at Kiama, 777 days post-release. Abbreviations used: degrees of freedom (df), Mean square (MS), F-ratio (F), row of mean square denominator (F vs), significance of the F test: P<0.01 (**), P<0.05 (*) and not significant (ns) (Cochran's *C* = 0.4742, ns; variances 4, df 11; conservative df from unbalanced data). Note, these data represent batch-tagged abalone only.

Source	df	MS	*F*	F vs
Location, L	3	787.05	8.72 **	2
Error	51	90.28		
Total	54			

6.4. DISCUSSION

Determining the value of a stock enhancement program requires the integration of a range of biological, economic and societal measures (Caddy & Defeo 2003, Hilborn 2004, Lorenzen 2005, Arnason 2008, Bell *et al.* 2008). Critical biological data needed to assess the value of a stock enhancement program are the survival and growth of released individuals. Estimates of survival and growth allow measures of the proportion and progression of released individuals into key life history (e.g. maturity) and fishery (e.g. MLL) stages. Together with economic data reflecting financial inputs and returns, these measures provide essential biological data for a bio-economic assessment of a stock enhancement program. An additional important objective of a stock enhancement program, and an important component in assessing its value, is the impact of releasing individuals on the wild population. The value of a stock enhancement program, even one that demonstrates high rates of growth, survival and economic return, may be substantially diminished if the release of individuals is detrimental to natural population growth that could otherwise result in comparable measures of value over time.

The objectives of the research presented in this chapter were to provide accurate long-term estimates of the survival and growth of batch-tagged *H. rubra* larvae and juveniles released to natural reefs, determine the impact of releasing juveniles on the abundance of wild *H. rubra* populations and provide a bio-economic analysis of a stock enhancement strategy for *H. rubra* in NSW. Results from the series of experiments outlined in this chapter demonstrated that substantial numbers of released individuals can survive within release locations for long periods of time (up to 916 days) and that rates of growth can result in the progression of released individuals (15 mm SL) to the commercial fishery within 2.5 - 4.5 years. However, rates of survival and growth varied substantially among releases and locations. Few releases provided long-term measures of survival and growth required to generate financially viable investment options for a stock enhancement program using parameters describing current economic conditions. Positive financial returns from a stock enhancement program for *H. rubra* in NSW became apparent only where long-term (>2 years) survival was >4% and this was achieved in association with above average rates of growth or moderate increases in economic returns (beach price >$AUD 34.kg^{-1}). The release of abalone to locations supporting depleted wild populations resulted in a significantly greater abundance of the total abalone population after >2 years. Further, the monitoring of released and wild abalone at release and control locations demonstrated that releases did not significantly affect the abundance of wild populations through time or limit wild recruitment and the growth of recruits to larger size classes in these populations.

6.4.1. Survival of released larvae and juveniles

The long-term survival of hatchery-reared and released abalone (this study, Table 6.4 and others, Table 6.10), and that of wild abalone (Shepherd & Breen 1992, Shepherd 1998, Worthington & Andrew 1998), varies substantially among locations and through time. Shepherd and Breen (1992) discuss an estimated average rate of survival through time based on published data for wild *H. rubra* (Figure 6.14, solid reference line). In that study, instantaneous rates of natural mortality ($M.yr^{-1}$) of larval and small juvenile *H. rubra* were high (M >5), and declined from approximately 2.5 at ~6 months of age, to 0.3 after 3 years and 0.2 over the following 2-3 years. Most estimates of the survival of released abalone reported in this study were lower than the expected estimates for wild abalone (Figure 6.14, points below solid reference line). Some estimates of survival suggested mortality rates more than twice that expected for wild counterparts (Figure 6.14, below dashed reference line). For example, the survival of released abalone at Red Point was many times lower than expected for wild abalone (Figure 6.14). This was probably related to the major habitat disturbance caused by rough seas within 10 days of this release. However, survival at three locations exceeded expected estimates for that of wild abalone after >2 years (Figure 6.14; Disaster Bay B & E and Kiama B).

The survival of *H. rubra* larvae released at three locations at Fingal Island - North provide the only quantitative estimates of the long-term (>1.5 years) survival of batch-tagged abalone larvae. These results demonstrated that the release of larvae can contribute to the total abalone population after >1.5 years, and that up to 0.03% of those released can survive (at an average length of 41 mm SL when recaptured). Low rates of settlement and estimates of survival within 56 days of the release of larvae were similar to those reported for the short-term (<60 days) survival of hatchery-reared and released *H. iris* (Schiel 1992), *H. rubra* and *H. laevigata* larvae (Preece *et al.* 1997). It is likely that the dispersal of larvae from release locations in this study would have been greater than that of studies by Schiel (1992) and Preece *et al.* (1997) as larvae in this study were not released into enclosures. Further, identification of recaptured abalone, through positive identification of the batch-tagged larval shell (this study), resolved the issue of estimates of survival being confounded by wild recruits, as reported by Preece *et al.* (1997), Shepherd *et al.* (2000) and Tegner (2000). It is likely that rates of emigration of juveniles from release locations through time, and sighting inefficiencies as reported by Preece *et al.* (1997) and Shepherd *et al.* (2000), would have contributed to more conservative estimates of the number of abalone surviving in this study (see discussion below for juveniles). The primary restriction on the use of larvae for stock enhancement, apart from low rates of survival, has been the costs related to their release which are associated with the methods used, i.e. the construction and deployment of shelters and the use of divers to direct the distribution of larvae on the reef. It is likely that minor modifications to the methodology used in this study, including a weighted hose

released from a vessel and towed through a release location to ensure an even distribution of larvae close to the reef, would provide a similar distribution of larvae to that obtained by a diver, without the associated operational cost. Further, high initial rates of mortality contributed to by visual predators may be substantially reduced by the release of larvae at night. However, the net effect on survival would require field testing with consideration of factors associated with differences in larval behaviour and a likely alternate suite of predators. Although these potential improvements in efficiency and survival are unlikely to make the release of larvae a viable stand alone option to successfully enhance local populations, they could contribute to their successful release and supplement an enhancement program where larval production is in excess of requirements.

The survival of *H. rubra* juveniles released to locations in this study displayed substantial declines soon after release and consistent declines through time. Long-term (>2 years) estimates of survival were <10% among locations (Figure 6.15). Of the twelve locations where released juveniles were surveyed for >2 years, three demonstrated rates of survival between 4-10% (Figure 6.15). These rates of survival are within the lower range of that reported for most other hatchery-reared haliotids released for >1 year (Table 6.10). For example, the survival of released *H. discus hannai* has been commonly reported in excess of 20% after >1 year and that of *H. iris* (released at 8 - 12 mm SL) ranged between 1.7 - 25.1% and 7.6 - 53.8%, from two independent studies, with survival >15% at the majority of locations (Table 6.10). The survival of *H. rufescens* in California has been variable among different studies. For example, Ebert and Ebert (1991) reported survival between 4.2 - 7.5% after 4 years, with some released abalone reaching the sport fishing legal size (>178 mm) within this time, while other studies have been less successful with survival after 12 months being reported between 0 - 1.2% (Table 6.10). The survival of *H. rubra* in excess of 4% after 2 years at locations in this study becomes increasingly important in consideration of the long-term financial viability of a stock enhancement program in NSW (see Section 6.4.4 Bio-economic assessment of a stock enhancement program).

The interpretation and comparison of estimates of abalone survival are complicated by a number of factors and their interactions that can change through time. Abalone utilise different habitats during their life history (Ault & DeMartini 1987, Sasaki & Shepherd 1996, Shepherd & Daume 1996, Seki & Taniguchi 2000) and have differential mortality among habitats that changes through time. This mortality can be manifest through the physical disturbance of habitat (unstable boulders) or exposure to different suites of predators, competitors (reviewed in Shepherd & Breen 1992) or commensals, such as urchins (Rogers-Bennett & Pearse 1998, Day & Branch 2000a). Further, other natural occurrences, such as disease and rates of emigration, as well as release protocols and sampling inefficiencies can affect survival and estimates of it.

Table 6.10 Summary of survival and growth for hatchery-reared juveniles released into natural habitat and recaptured after >1 year (not including this study). Japanese fishery recaptures have not been included in this table (see reviews in Masuda & Tsukamoto 1998, Hamasaki & Kitada 2008). [†]sampling time is estimated; [*]survival (%) is generated from sighted and non-sighted individuals at each sample time.

Species Reference	Length at release (mm) (range)	Number released (x 10^3)	Area of location (m^2)	Number released.m^{-2}	Time sampled post-release (d, m, y)	Survival (%)	Instantaneous mortality ($M.yr^{-1}$)	Growth ($\mu m.day^{-1}$) [a] ($mm.yr^{-1}$) [b]	Habitat type	Comment
H. discus hannai										
Honma & Iioka (1980)	20 - 45				2 (y)	51 - 65				in McCormick (1994)
Takeichi (1981)	20 - 50				2 (y)	25 - 50				in McCormick (1994)
Miyamoto *et al.* (1982)	<10				491 (d)	10.0	1.7			in Tegner & Butler (1985)
	>22				491 (d)	31.0	0.9			
Sakamoto *et al.* (1982)	(15 - 50)				2 - 3[†] (y)	13.0 <10	0.7 - 1.0			in Tegner & Butler (1985)
Saito (1984)	24.8 (8 - 50)	19.40	50	388.0	364 (d)	10.0 <22 mm	2.3	~20 [b]		Increased survival with length
						23.0 >22 mm	1.5			Seasonal variation in growth
					491 (d)	8.0 <22 mm	1.9			
						31.0 >22 mm	0.9			
Seki & Taniguchi (2000)	24.5 (13 - 35)	100.00	15		853 (d)	26.7	0.6	75 [a]	'cobble'	Survival varied among habitats
	16.5	26.00			518 (d)	37.0	0.7	11 - 181 [a]	'cobble'	Seasonal variation in growth
H. fulgens										
Guzman del Proo *et al.* (2004)	23.5 (16 - 49)	0.53	200	2.7	1 (y)	12.67[*]	2.1	48.1 [b]	intertidal	Compare release and wild
					2 (y)	5.67[*]	1.4		boulders	Under-estimated survival
					3 (y)	1.13[*]	1.5	28.1 [b]		Sighting efficiency
H. gigantia										
Inoue (1976)	30 - 100				1 (y)	>60.0 >40 mm				in Tegner & Butler (1985)
H. iris										
Schiel (1993)	8.6 (3 - 13)	10.00	507	19.7	23 (m)	53.8	0.3	22.6 [b]	boulders	
	12.2 (3 - 22)	9.90	280	35.4	12 (m)	9.9	2.3	21.0 [b]	solid, boulders, sand	Habitat change (sand)
	12.2 (5 - 22)	9.85	150	65.7	19 (m)	7.6	1.6	14.0 [b]	solid, boulders	Emigration (50 m)
	11.8 (5 - 22)	9.85	340	29.0	19 (m)	24.2	0.9	23.8 [b]	solid, boulders	Higher survival in 2nd survey
Roberts *et al.* (2007)	11	20.00	400	50.0	17 (m)	1.7	2.9	33.4 [b]	boulders	Area derived from densities (50 m^{-2})
	10	6.52	130	50.0	20 (m)	7.3	1.6	29.8 [b]	boulders	Habitat change (sand)
	10	10.01	200	50.0	20 (m)	25.1	0.8	30.1 [b]	boulders	
	11	5.00	100	50.0	17 (m)	18.7	1.2	25.0 [b]	boulders	
	10	2.61	52	50.0	20 (m)	16.2	1.1	25.1 [b]	boulders	
H. midae										
Sweijd *et al.* (1998)	8.2 - 14.8	0.5 - 0.8			6 (m)	25.6 - 39.2	1.9 - 2.7	13 - 17 [b]	sheltered, boulders	Cumulative survival at 18 months
H. rubra										
Shepherd *et al.* (2000)	12.1 (7 - 22)	0.30	672		12 (m)		1.8	30.3 [b]	boulders	Number released inferred from densities
H. rufescens										
Tegner & Butler (1985)	45 - 71 (40 - 80)	0.67	625	1.1	1 (y)	1.1 - 1.2	>4.4	30 [b]	boulders, flat rock	Multiple handling, predation
Ebert & Ebert (1991)	(10 - 50)	7.40	1810		13 (m)	>8.9			solid, boulders	Release shelters, emigration
			7882		4 (y)	4.2 - 7.5		<39.6 [b]		few legal size, bar-cut fishing mortality
Rogers-Bennett & Pearse (1998)	8.2	5.0 - 10.0	60 (720)		1 (y)	0.0 - 0.17	>6.4	~15 [b]	boulders	Release shelters and hand planting
					2 (y)	0.0 - 0.21	>3.1	~15 [b]		Increased survival with urchins

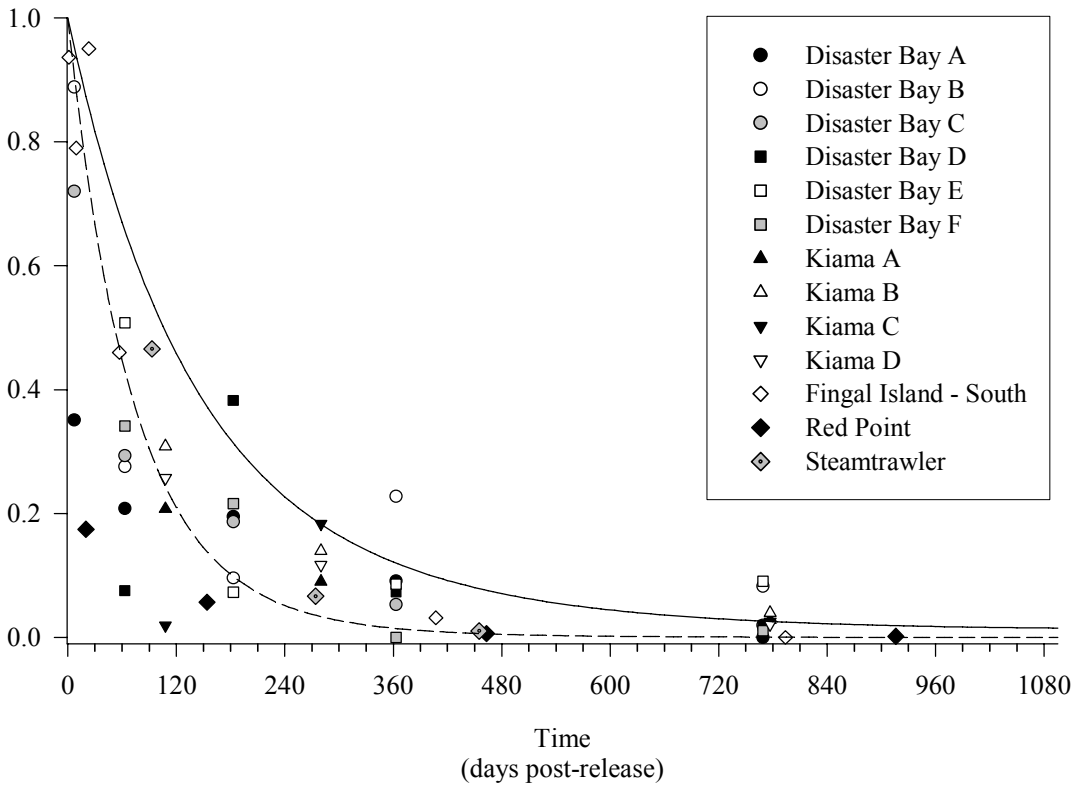


Figure 6.14 Proportion of released juvenile abalone surviving at locations through time. Solid line provides a reference of a linear decline in *M* from 2.5 to 0.3 from 0 to 1 095 days for wild *H. rubra* (adapted from Shepherd & Breen 1992). Dashed line provides a reference of twice the *M* of the solid line. Note, the curved lines represent linear declines in *M*, but are plotted as the proportion of abalone surviving through time.

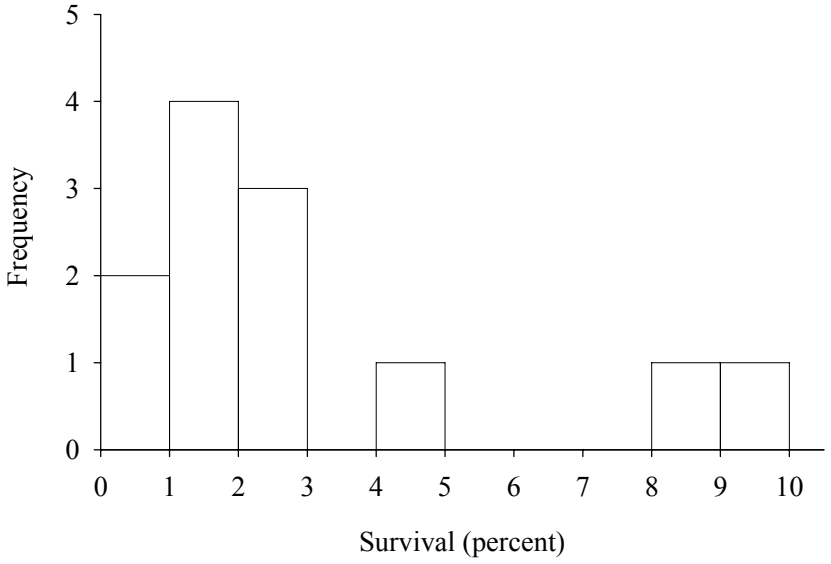


Figure 6.15 Frequency distribution of the long-term (>2 years) survival (percent) of released juvenile abalone at the twelve locations in this study.

Natural occurrences including disease and substantial habitat disturbance, as a consequence of rough seas, likely contributed to the mortality of released abalone in this study. For example, the number of released abalone at Fingal Island - South declined from ~2500 to zero between 407 and 794 days post-release. Coincident with this decline was a mortality event resulting in a decline in local wild populations of >90%, associated with high levels of infection with *Perkinsus olseni* (Anon 2005c). Further, despite declines in local populations of abalone along the NSW central coast that have been associated with infections with *P. olseni*, similar disease-related mortality events have generally been rare in wild populations. However, similar events have been documented for 'withering syndrome' in California, USA (Haaker *et al.* 1992, Steinbeck *et al.* 1992), *P. olseni* in South Australia (Goggin & Lester 1995) and abalone viral ganglioneuritis in Victoria, Australia (Appleford *et al.* 2008).

The large-scale disturbance of habitat and the negative effect this has on the survival of released abalone was evident in this study and has been described in a number of similar studies (Tegner & Butler 1989, Schiel 1993, Osumi 1999, Roberts *et al.* 2007b) and also for wild populations (Poore 1972, Sainsbury 1982, Shepherd & Breen 1992). The survival of <20% of released abalone within 20 days of release at Red Point (this study) was likely influenced by rough seas disturbing boulder habitat and crushing released abalone (personal observation), and subsequent partial sand inundation of the location between 0.5 - 1 year after release. Moreover, differences in the density of released abalone between disturbed boulder habitat and solid habitat within release locations at Disaster Bay and Kiama were demonstrated in this study, and the major disturbance of boulder field habitat, by rough seas, at these locations undoubtedly contributed to reduced rates of survival.

The small scale (<10 m) distribution of abalone among habitats can substantially affect estimates of survival of released abalone (this study, Seki & Taniguchi 2000, Shepherd *et al.* 2000). Dixon *et al.* (2006) found significantly greater survival of released *H. laevigata* among boulder habitat consisting of 2 layers of boulders rather than 1 layer and attributed this difference to cryptic habitat availability. In this study, solid habitat supported significantly greater densities of released abalone than did substantially disturbed boulder habitat, a likely consequence of mortality caused by crushing (personal observation) or movement of abalone into solid habitat. Differences in density among habitats at small spatial scales and the implications for estimates of survival requires further investigation. It is possible that solid habitat, that provides a more stable habitat to that of boulder fields, may support a greater density of abalone, even when boulder fields are not disturbed. Alternatively, differences in density may be explained by abalone moving, or being redistributed by local currents into solid habitat after they detach from disturbed substrata (personal observation, Momma *et al.* 1980, Hooker 1988; cited in Osumi 1999). If the former is the case, estimates of the total number of abalone and rates of survival

reported in this study where solid habitat contributed a substantial proportion of a release location, are more conservative than reported. Further, differences in carrying capacity of different habitats and the densities with which some juveniles were released to locations in this study could have resulted in rates of growth and survival that were lower than could have been achieved. Lower densities within locations through time could result in a mean increase in the rates of survival and growth (Day & Fleming 1992, Shepherd & Breen 1992, Shepherd 1998, Dixon & Day 2004).

Emigration of individuals from the survey area will contribute to declines and reduced accuracy in estimates of survival within the survey location through time. A contribution to substantial declines in survival has been attributed to the emigration of abalone after release (Shepherd 1986a, Ault & DeMartini 1987, Emmett & Jamieson 1989, Ebert & Ebert 1991, de Waal *et al.* 2003). Moreover, where resources to accurately determine rates of emigration limit their estimation, estimates of survival through time are likely to be increasingly conservative (this study, Tegner & Butler 1989, Guzman del Proo *et al.* 2004). Despite criteria for the selection of release locations used in this study being the presence of some form of natural habitat boundaries to limit emigration, such as Barrens and vertical walls extending to the intertidal, it is likely that these boundaries did not entirely prevent it (Werner *et al.* 1995). However, habitats within release locations did support populations of released and wild abalone for long periods of time and *ad hoc* observations of the area surrounding release locations did not indicate high rates of emigration from these locations. Further, in this study devices holding juveniles were placed within the boundaries of release locations so that initial movements would have been within the location. Sampling designs incorporating methods to explicitly estimate rates of emigration from locations would improve the precision and accuracy of survival estimates where individuals move from the survey area (Schwarz & Seber 1999, Schwarz 2005). Glazer (2005) describes the application of estimating emigration of a slow-moving benthic marine mollusc (*Strombus gigas*), including probabilities of emigration from the survey area, to provide more accurate and greater estimates of survival (by 20%) than that generated by recovery data. More accurate and greater estimates of survival through the application of these techniques would substantially benefit the assessment of value of a stock enhancement program and potentially have a substantial effect on estimates of its financial viability.

Processes involved in the release of individuals to natural reefs including handling and transport would have affected rates of survival, particularly within short periods of time after release. However, the development and adherence to protocols of handling, transport and release of abalone described in earlier chapters would have minimised these impacts. Moreover, there was no significant difference in the long-term survival of juveniles released in single dense groups compared with that of more numerous less dense groups after >2 years. Similar results were

reported by Goodsell *et al.* (2006), although their study only spanned a short period of time (1 month) and employed surveys that did not disturb the substrata. Further, preliminary results of more diffuse groups providing greater rates of survival from the experiment done as part of this study and reported by Heasman (2006), highlights the necessity to carry out long-term studies on the effect release procedures have on survival (and growth, see below). Basing decisions regarding the logistics and management of a large-scale program or future research directions on relatively short-term results could result in substantially increased and unnecessary costs.

Estimates of survival would also have been affected by the efficiency of the sampling methods used and differences in sighting efficiency through time as abalone increased in size. Shepherd (1990, 1998), Guzman del Proo *et al.* (2004) and Dixon *et al.* (2006) describe correction factors for survival estimated from abalone surveyed at more than one time, and where sampling methods included thorough investigation of the habitat. The magnitude of these corrections range between 1.25 - 2.8. Where sampling methods are less accurate, such as surveys where habitat is not disturbed, survival would be underestimated by factors greater than described by these authors. Similarly to those studies where correction factors were used, long-term sampling at all locations in this study employed methods that disturbed the substrata (as recommended from outcomes in Chapter 5). However, as abalone were not individually tagged in this study no estimate of sighting efficiency was possible and no multiplier was used to modify estimates of the number of abalone sampled within release locations. Estimates of survival in this study are therefore considered conservative.

A number of other processes, associated with hatchery-reared and released individuals, are likely to have contributed to the survival of released abalone in this study. For example, others have described the potential impact of naivety on the survival of released abalone. Schiel and Welden (1987) demonstrated that hatchery-reared abalone displayed slower responses to seek shelter and had higher rates of mortality associated with subsequent predation, although no significant differences were observed between hatchery-reared abalone of different size. Osumi (1999) described the potential to reduce the impact of some hatchery induced behaviours on the initial survival of released abalone through the use of different tanking systems prior to release. No behavioural modifications were attempted on the abalone released in this study. However, the release of abalone in devices that provided initial protection from predators and reduced handling stress and subsequent associated mortality, would limit the effect of these processes on early mortality. Further, the transition of early juveniles (~2 mm SL) from a diet of diatoms to artificial feed, that provide for increases in juvenile production (Heasman *et al.* 2004), may have affected rates of survival in this study. The transition to an artificial diet in the hatchery at an early age may limit the physiological and physical development, including a fully developed digestive tract, of early juveniles and limit their ability to gain essential nutrients from a natural

diet once released (Kemp 2001). The effect of this husbandry practice on the survival and growth of abalone released in this study is unknown. It is possible that early juveniles require longer times on diatom diets after settlement as larvae, at the expense of production efficiency, or improved artificial diets may be required to provide fully functional physiological and physical development of juveniles. Improved husbandry techniques could result in increased rates of survival and growth of released abalone, and greater than reported in this study.

6.4.2. Growth of released larvae and juveniles

Rates of growth reported for released *H. rubra* in this study showed substantial variation at spatial scales ranging from 100s m to 100s km. This variation in growth at different spatial scales is typical of that reported for wild *H. rubra* populations (Day & Fleming 1992, Worthington *et al.* 1995, Prince 2004, Saunders & Mayfield 2008). Moreover, the variation in growth at spatial scales reported for *H. rubra* is commonly reported among other populations of haliotids, with many factors and their interactions proposed to explain this variation. Factors reported to contribute to this variation include differences in the supply and quality of food, physical oceanography, including wave energy and water temperature, season, population density, ontogeny and genetics (Shepherd 1973, Shepherd & Laws 1974, Shepherd & Hearn 1983, Emmett & Jamieson 1988, McShane *et al.* 1988b, Prince *et al.* 1988b, Day & Fleming 1992, Nash 1992, McShane & Naylor 1995a, Tarr 1995, Worthington *et al.* 1995, Worthington & Andrew 1997, Troynikov *et al.* 1998, Tegner *et al.* 2001, Dixon & Day 2004, Prince 2004, Naylor *et al.* 2006a).

Worthington *et al.* (1995) reanalysed and compared published annual growth rates for wild *H. rubra* spanning their geographic distribution (Shepherd & Hearn 1983, McShane *et al.* 1988b, Prince *et al.* 1988b, McShane & Smith 1992) at three length classes (65, 90 and 115 mm SL), and reported annual growth rates in the range 7.8 - 38.0, 4.3 - 25.7 and 0.8 - 13.3 mm.yr^{-1} among these three length classes, respectively. Mean rates of annual growth of released *H. rubra* that were recaptured after >1 year in this study ranged from 17.8 - 47.9 mm.yr^{-1}, with mean total shell lengths in the range 32.0 - 107.3 mm. At spatial scales of <5km (this study) rates of annual growth of released abalone varied significantly after 2 years among locations at Kiama, ranging between 22.9 and 30.2 mm.yr^{-1}, with the shell lengths of individuals ranging between 62 - 77 mm. These results fall within the range, and are among the higher estimates of growth, for wild *H. rubra* of this length as described by Worthington *et al.* (1995). However, mean annual rates of growth from individuals recaptured after 1 (~60 mm SL) and 2 (~100 mm SL) years at Disaster Bay (this study) were 43.3 and 41.8 mm.yr^{-1} among locations, respectively. These rates of growth were the highest recorded in this study and the highest among other studies for wild *H. rubra* (Worthington *et al.* 1995).

Rates of annual growth reported for hatchery-reared haliotids released for >1 year commonly range between 20 - 30 mm.yr^{-1}, although some studies report rates >35 mm.yr^{-1} (Table 6.10; Seki & Taniguchi 2000, Guzman del Proo *et al.* 2004). Notably, Ebert and Ebert (1991) describe annual growth rates of >30 mm.yr^{-1} for four consecutive years such that a proportion of released *H. rufescens* had recruited into the legal length for sport fishing in northern California (178 mm; Table 6.10). Similarly, in this study a single *H. rubra* released at 15 mm SL at Disaster Bay and recaptured after 2 years had reached 116 mm SL, 1 mm greater than the MLL for the NSW commercial fishery at the time of recapture. Further, abalone released in concentrated groups among locations at Disaster Bay demonstrated significantly faster growth and larger size (mm SL) >2 years post-release, compared with those released in a diffuse array. It is possible that initially high numbers of abalone within a small area may proffer some growth advantage through individuals trapping drift algae that can provide food to others within close proximity.

Comparisons of rates of growth among locations separated by large (>5 km) spatial scales in this study were confounded by the release of different batches of abalone. This was a necessary component of this study as batches of abalone released among local populations (<5 km apart) were required to be produced from local broodstock, to provide some protection of the genetic integrity of local wild populations. Batches of abalone released to single locations were also released at different times of the year, further adding to difficulties in comparisons. Temporal comparisons among locations were not done as measures of individuals at different times were not assured to be independent and rates of growth can vary though time in association with many factors including seasons, water temperature and food supply (Shepherd & Hearn 1983, Day & Fleming 1992, Troynikov *et al.* 1998). Despite these restrictions, the rates of annual growth of released *H. rubra* in this study were generally higher than reported for similar lengths in wild populations. This was also reported by Guzman del Proo (2004) for released *H. fulgens*, and similar to those of other species of haliotids released at similar lengths to natural habitats and recaptured after >1 year (Table 6.10). It is possible that rates of growth estimated from wild populations underestimate rates of growth at smaller lengths. This is likely as samples obtained to estimate growth often under-represent smaller, cryptic abalone that are difficult to sample and growth of larger individuals may misrepresent possibly faster rates of growth in juveniles before they approach maturity (Prince 2004). The use of released juveniles may provide an opportunity to test hypotheses regarding processes affecting small-scale differences in patterns of growth and other processes affecting wild populations (Miller & Walters 2004).

The average rates of growth described among locations in this study, in association with data on the maturity of *H. rubra* in NSW (Worthington & Andrew 1997), indicate that abalone released at 7-15 mm SL would be expected to reach maturity (>90 mm SL) after 2 - 3 years, and the minimum legal length of 117 mm (as at July 2008) after 2.5 - 4.5 years. These predictions have

substantial implications for the decisions required to invest in a stock enhancement program for *H. rubra* in NSW, Australia, the management objectives of such a program and an assessment of its value (discussed below).

6.4.3. Impact of stock enhancement on wild populations

This is the first study to explicitly test the impact of releasing abalone on the abundance of wild populations among multiple release and control locations (Experiment 3, this chapter). A small number of studies that have released marine animals for stock enhancement have included untreated or treatment control locations to assess differences in yield or production of the target species (wild and released animals combined; Leber 1999, Crowe *et al.* 2002). Further, there is continued debate regarding the value of stock enhancement due to the uncertainty of its contribution to populations. Numerous studies have described changes in yield and ratios of released and wild conspecifics in commercial landings as demonstration of the increased value of releasing individuals (Kitada 1999, Wertheimer *et al.* 2004) or alternatively, the failure of doing so (Svåsand *et al.* 2000, Hilborn 2004). The importance of assessing the impact of releasing individuals on the wild population through an experimental framework, including untreated controls, has been overlooked in most studies describing the release of individuals, including those releasing abalone (Bell *et al.* 2005), and has limited the interpretation of their findings. For example, in most instances it is unknown whether released individuals augment or replace wild recruits (Hilborn 2004, Bell *et al.* 2005, Lorenzen 2005, Bell *et al.* 2006).

Populations of wild *H. rubra* at multiple locations within the area of Kiama, NSW, had been regularly surveyed as part of a fishery-independent stock assessment program, by the NSW Department of Fisheries, for many years (Andrew *et al.* 1996, Worthington *et al.* 2001). These surveys quantified the decline in abundance of wild populations of *H. rubra* to below 25% of previously recorded levels of abundance over a period of 4 years from 1995 (Worthington *et al.* 2001, Anon 2005c). Population declines in this area were coincident with positive results of *P. olseni* infection within wild populations after these declines were first detected (Worthington *et al.* 2001, Anon 2005a). Subsequent to these findings and as an outcome of quantified declines in local wild populations, large areas of coast within the vicinity of Kiama were closed to fishing by all sectors (Worthington *et al.* 2001). Testing of wild populations after initial positive results for *P. olseni* indicated low levels (5%) of infection at two of five locations tested within the Kiama area (NSW Fisheries unpublished data).

The release of abalone into multiple locations at Kiama and the monitoring of released and wild abalone among four length classes within release and control locations in this study (Experiment 3) has provided the first investigation into the impact of releasing abalone on the abundance and

persistence of wild populations. The release of 9 000 (14 mm SL) abalone into each of the release locations resulted in a significantly increased total abundance of abalone after >2 years, with the average total number of abalone within release locations being >200% of that in control locations. Moreover, released abalone contributed between 42 - 58% to the total population within release locations. Surveys of four length classes of wild abalone within release and control locations demonstrated the progression of a cohort of wild recruits that dominated the 30-60 mm length class at 280 days post-release, and then the 60-115 mm length class at 777 days post-release. Consistent patterns in the abundance of these length classes among release and control locations support the hypothesis that the release of abalone does not significantly affect natural population growth as determined through wild recruitment and growth. An important consideration of this finding is the initially depleted state of natural populations prior to the release of abalone. A number of reviews of stock enhancement studies have emphasised the need to complement natural population growth processes and have highlighted the likely increased value of a program where releases are done among natural populations that have been depleted to very low levels and where depensatory population growth processes are likely to operate (Hilborn 2004, Leber 2004, Lorenzen 2005, Bartley & Bell 2008, Lorenzen 2008). The persistence of natural recruits to populations among release locations at Kiama was only possible through the availability of natural resources not capitalised by released individuals, i.e. the carrying capacity within release locations was not sufficiently limited by the introduction of 9 000 (14 mm SL) abalone. Results from this study demonstrate the value of a stock enhancement program to complement natural population growth processes and help rebuild depleted abalone populations.

6.4.4. Bio-economic assessment of a stock enhancement program

The bio-economic analysis of a long-term (10 year) theoretical release program was done using: 1) estimates of survival and growth derived from releases described in this study; 2) parameters for alternate measures of survival (Shepherd & Breen 1992); 3) intermediate measures of growth (Worthington *et al.* 1995); 4) production costs from commercial farms and the literature (Hart *et al.* 2007); 5) economic data from the NSW abalone industry; and 6) opportunity costs derived from the literature (Caddy & Defeo 2003). Positive financial investment opportunities were gained using current estimates of financial inputs and returns where rates of survival and growth of released abalone were above the average of those measures obtained in this study. Similar positive returns were reported by Schiel (1992) for *H. iris*, where rates of survival (24.3% after 3 years) were derived from above average results gained in field releases and subsequent survival was optimistically set at an instantaneous rate of natural mortality (*M*) of 0.1 (from Sainsbury 1982). Less optimistic values of *M* and lower survival within 3 years indicated likely economic

losses that were sensitive to rates of survival and growth (Schiel 1992). Roberts *et al.* (2007b) demonstrated substantial economic benefit from the release of *H. iris* using average rates of survival from field studies (10 - 15%) and the same optimistic rate of *M* (0.1) offered by Sainsbury (1982), and noted the need to address the potential of released individuals replacing rather than supplementing wild recruits. There was little evidence for this in this study (Experiment 3).

In this study, scenarios describing financial returns greater than those from an alternative investment opportunity returning 10% p.a. were obtained after 10 years, where 7 mm abalone were released and demonstrated high rates of growth (G_{III}) and intermediate rates of survival (S_{II}), or where rates of survival were high (S_{III}) irrespective of growth rates. Further, positive rates of NPV within the first 5 years of the program, i.e. whilst annual investment was still being made, indicated that the continuation of the investment strategy beyond the 5 year period described in this study would provide increasingly positive NPV through time.

The factors investigated in this bio-economic assessment all played crucial roles in the projected financial outcomes for the scenarios described. Further, interactions among these factors substantially altered the economic outcomes. For example, increased rates of growth reduce the time taken for individuals to reach the minimum legal length and this reduced time translated to a greater proportion surviving, increased harvest, greater annual cash flow and greater rates and final values of NPV. A major factor affecting financial return was the rate of survival. Without adequate survival to harvestable size, no increase in financial return (reduced production costs or increased beach price) would provide financial benefit greater than that of an alternative investment returning 10% p.a. (i.e. NPV >0). Where released abalone did survive to the minimum legal length, increased rates of growth reduced the time taken to harvestable size and resulted in increased NPV.

The deterministic model used in this study provides an informative guide, at a relatively coarse financial scale, to gauge the likely financial feasibility of a stock enhancement program for *H. rubra* in NSW. A number of assumptions were made to generate the outputs reported. Finer resolution of these financial outputs could be gained by adding complexity to the model to describe additional biological variation and financial inputs not included in this study. The model used in this study did not include measures outlined in the points below (an account of these issues is described in the following paragraphs of this section):

1. cost of releasing or harvesting abalone or monitoring survival prior to harvest.
2. added risk from variation in yield attributable to factors including disease or the loss or reduction of habitat or low rates of growth at locations.

3. initial capital investment and additional costs in production associated with protocols to ensure genetic diversity is maintained within the target population and that released individuals are tested to ensure against the introduction of known diseases.
4. impacts of positive or negative population growth or reduced fitness of future populations. For example, the contribution released individuals may make to future recruitment or impacts on carrying capacity limitation, density-dependent effects on survival or growth, or potentially reduced genetic diversity of wild populations.
5. costs associated with measuring assessment targets of non-financial returns including those of societal, political and environmental requirements.
6. indirect benefits attributable to stakeholders, including secondary economic benefits to the commercial sector, such as adjusted asset value of shares, increased value to the recreational and indigenous fishing sectors and added societal value.

Costs of releasing, harvesting and monitoring abalone were not included in this bio-economic assessment on the understanding that releasing abalone to natural reefs in NSW would provide increased security to all stakeholders in the resource, with financial benefit flowing primarily to the commercial harvest sector. As such, these costs were considered to be an in-kind contribution from stakeholders likely to be financial beneficiaries.

This model did not account for stochastic variation in survival and growth. Spatial and temporal variation in rates of survival and growth, and the subsequent financial impacts of this variation on a stock enhancement program, require investment decisions regarding an acceptable level of risk to be offset against costs of increased replication. For example, a greater number of enhanced locations would reduce the likelihood of a substantial proportion of them returning low rates of survival due to habitat loss, low rates of growth, or disease. However, greater levels of replication would require a greater level of investment. Further, the nature of the population depletion will affect the level of risk associated with reduced survival. For example, where populations have been depleted by disease it is likely that there would be a greater level of risk of reduced survival, unless adequate testing can provide assurances of the remaining population being disease free. Alternatively, investment could be made in pilot scale experiments to obtain some measure of these factors and hence reduce the level of risk associated with their variation. However, pilot scale experiments can not be used to assess some levels of impact, for example measures of competitive interactions within the broader ecology of the system (Hilborn 2004).

The initial capital investment required to build a hatchery to supply abalone to an enhancement program would be substantial. Further, the development of a breeding and rearing program to support a management strategy to enhance local populations of *H. rubra* would require the

development of protocols for the maintenance of genetic diversity at the scale of local wild populations (Ward 2006, Bartley & Bell 2008) and the integrity not to introduce or increase the prevalence of disease in local wild populations (Caddy & Defeo 2003, Bartley *et al.* 2006). Whilst initial capital investment costs have been assumed to be incorporated into the commercial price paid mm^{-1}, it is likely that existing protocols within commercial hatchery systems, designed for the maximum production of abalone for sale are ill-designed for requirements of genetic diversity and integrity. Production costs of individual abalone, based on these existing commercial protocols are likely to reflect optimistic values for a local population enhancement program.

The release of abalone for the stock enhancement of wild populations has been made under the assumption that natural stocks have been depleted to levels below those where natural processes of population growth are likely to be affected by the addition of individuals and where released individuals contribute to a substantial proportion of recruits (Bell *et al.* 2005, Lorenzen 2005, Bell *et al.* 2006). Where this occurs, and the magnitude of released individuals is below previous abundance levels, there would be a reduced likelihood of exhausting the carrying capacity of existing habitats and of having adverse impacts on population growth processes associated with density dependence or other depensatory processes affecting population growth. However, the potential genetic impacts of releases made under the circumstances of severely depleted natural populations requires carefully considered stock management (Lorenzen 2005, Ward 2006, Le Vay *et al.* 2007, Bartley & Bell 2008).

Important factors that must be considered to complete a thorough assessment of a stock enhancement program include those stemming from social and political motivations and environmental requirements including genetic and ecological impacts on the target species as well as the broader ecological environment (Blankenship & Leber 1995, Munro & Bell 1997, Caddy & Defeo 2003). The costs associated with assessing the objectives and targets of these factors will be a component of any stock enhancement program (Arnason 2008, Lorenzen 2008). The development of these objectives requires inclusive and transparent interaction among stakeholders and investors, so that there is confidence that assessment targets of non-financial, as well as financial returns, are adequately addressed. A socially, politically and scientifically acceptable level of difference in the genetic integrity of the original population against a future enhanced population and temporal assessment of the abundance of a suite of co-habiting organisms would enable targets against environmental variables to be measured. In addition, minimum survival targets for progressive size classes through time would enable an assessment of the financial value of releases against investment prior to commercial harvest, and minimal commercial harvest targets would support the financial feasibility of a program's continuation.

6.4.5. Implementation of a stock enhancement program for **H. rubra** *in NSW*

The decisions required to invest in a stock enhancement program need to be made against a fully informed understanding of the current biological and economic status of the extant populations and in light of current, complementary and alternative management arrangements that may provide comparative increases in value (Hilborn 1998, Caddy & Defeo 2003, Caddy & Agnew 2004, Bell *et al.* 2005). A management group consisting of representatives of stakeholders, guided by independent and informed specialists, would be required to determine a course of action through the development or modification of a management plan to add value to the existing wild abalone resource (Bell *et al.* 2008). The inclusion of a stock enhancement program in such a plan would require a series of decision rules to be considered to initiate and implement such a plan in the first instance. For example, there would be increased risk to the success of a program where local population depletions have been the subject of disease rather than over-fishing. The decision to implement a stock enhancement program at local scales where disease has occurred would, at a minimum, require the testing of remaining populations with resultant negligible or nil infection, prior to any introduction of hatchery reared individuals. Further, it is critical that objectives of a program be explicitly described and understood by all stakeholders, and a series of targets developed, against which the objectives can be assessed, the program evaluated and investment or disinvestment decisions made within specified timeframes. Targets would likely include levels of increased abundance or harvest within a timeframe and other measures of financial and ecological importance. In concert with the development of a program for stock enhancement there is a need for policy development to provide an accurate measure of risk to investors and stakeholders of the outcomes delivered through the success of a stock enhancement program (Caddy & Defeo 2003, Lorenzen 2008, Tringali *et al.* 2008).

The cost of a stock enhancement program would be minimised where it does not function independently of other potentially collaborative, complementary and supportive ventures. The cost of releasing individuals from a hatchery would be significantly reduced through a cooperative arrangement with stakeholders likely to benefit from its success and those that have invested in the capital and human resources required to support the release of hatchery-reared stock (Bell *et al.* 2008). The most obvious of these parties for the stock enhancement of *H. rubra* in NSW are the commercial harvest and aquaculture sectors. The collaborative involvement of these sectors, with their complementary wild harvest operations and hatchery production, would provide substantial financial and logistic benefits to a program whilst reducing the need for direct cash investment. Other substantial stakeholders are likely to include those managing the public value of the resource, such as all levels of government. These stakeholders may also complement the necessary commercial costs of a stock enhancement program, given the development of objectives and outcomes that support many of the primary principles of their

management. For example the 'objects' of the NSW Fisheries Management Act 1994 are to conserve, develop and share the fishery resources of the State for the benefit of present and future generations and to conserve populations and ecological communities of fish, promote viable commercial fishing and aquaculture industries and promote recreational fishing opportunities. However, it is likely that investment and financial commitment from stakeholders likely to benefit from successful enhancement would be required to facilitate financial support from public funding to initiate a program that delivers outcomes to support public institutional objectives of population persistence (Bartley & Bell 2008).

The current 'Environmental Impact Statement on the Abalone Fishery in NSW' has afforded the scope to undertake stock enhancement as an objective under the goal to 'Maintain or rebuild the biomass of abalone to ensure stock sustainability', noting the need for consideration of articles in the Fisheries Management Act 1994 and the need for an environmental impact statement for such a venture under the Environmental Planning and Assessment Act 1979 (Anon 2005b). Current management of the NSW Abalone Fishery has seen increased spatial restriction within the commercial and recreational fisheries, and consecutive and substantial reductions in the total allowable commercial catch (Anon 2005c, 2008b). More recent changes have included increases in the state-wide MLL and attempts at adaptive management strategies to obtain information to inform the status of wild stocks (Anon 2008a, 2008b). The severely depleted condition of many local populations and continued limited recruitment (Anon 2005c, 2008b) provides strong support for the likelihood of a stock enhancement program to deliver increased value to these populations and the fishery.

6.4.6. Conclusion and recommendations

Long-term (>2 year) estimates of survival and growth from the release and monitoring of abalone in this study demonstrated that released abalone can enhance local populations and that they contribute to the mature population within 2 - 3 years, and to the legally harvestable population within 2.5 - 4.5 years. The release of abalone among replicated locations did not have a negative impact on the abundance of depleted extant populations, compared with control locations. Moreover, natural recruitment and the progression of wild recruits to larger length classes within wild populations was unaffected by the introduction of hatchery-reared abalone to locations more than 2 years earlier. Financial returns in excess of an alternative investment strategy returning 10% p.a. were demonstrated for the harvest of released abalone using a deterministic bio-economic model (including important investment assumptions – Section 6.4.4) where current economic circumstances were used with rates of survival and growth above the average of those described in this study. Results from the release of juveniles demonstrated that stock enhancement can provide an alternative and complementary management option where

extant populations have been depleted and would increase the value of some of the local populations of *H. rubra* in NSW, Australia.

Quantifying the effect of some pre- and post-release factors on the long-term survival and growth of released abalone would provide more accurate and likely greater estimates of both of these critical parameters for the assessment of the value of a stock enhancement program. For example, confirmation that intensive hatchery production protocols do not compromise the fitness of abalone for release to natural reefs and the lessening of potential behavioural weaknesses from hatchery-reared individuals would provide greater confidence in the investment in this technology for stock enhancement. Further, estimates of rates of emigration and sighting efficiency through time would improve estimates of survival pre-harvest and improve the potential financial feasibility of a stock enhancement program.

Spatial and temporal variation in demographic parameters, including the survival and growth, of released individuals restricts the use of many generalities gained by other than specific local scale experiments. However, studies of the release of all haliotids have reported habitat disturbance at some spatial scale as having a negative effect on survival. A greater level of consistency and increased estimates of long-term survival would be achieved where abalone were released to reef that is temporally stable and not prone to physical disturbance, although this would limit the ability to obtain accurate estimates of survival prior to harvest. Further, the nature of local population decline would require consideration with respect to the projected and realised success of an enhancement program. Populations depleted through disease would be less likely to deliver similar relative value from an enhancement program than those depleted through over-fishing.

In conclusion, the decision to implement a stock enhancement program for *H. rubra* in NSW needs to be made among well informed representatives of all stakeholders in the resource with a thorough understanding of the current biological structure of the populations and economic status of the fishery. Further, this decision needs to be made in light of current and alternative management options available to deliver increased value to these populations and the fishery. Despite potential financial benefits demonstrated by the bio-economic assessment in this study, the greatest value of a stock enhancement program for *H. rubra* in NSW will be gained where the primary management objective is the rebuilding of depleted natural populations, i.e. 'restocking', rather than optimising commercial yield through releasing individuals to overcome recruitment limitation, i.e. 'stock enhancement', or to harvest released individuals at a larger size, i.e. 'sea-ranching' (as defined by Bell *et al.* 2008).

7. GENERAL DISCUSSION

The research described in this thesis has provided the methodology, baseline targets and recommendations for the implementation of a stock enhancement management strategy for local populations of blacklip abalone in NSW, Australia. It comprises the first body of work to develop and test processes and their application for the stock enhancement of local populations of *H. rubra* and one of the first to undertake long-term monitoring of released individuals, demonstrating survival to commercial size. The principal findings were that the release of hatchery-reared *H. rubra* juveniles can enhance local depleted populations and that the greatest value of a stock enhancement strategy is likely to be gained where its primary objective is rebuilding depleted populations, rather than optimising commercial yield through the release of individuals to overcome recruitment limitation or to harvest released individuals at a larger size. Moreover, low levels of long-term survival of released larvae suggest their large-scale release is unlikely to add substantial value to a stock enhancement program and should only be included in a program where their production exceeds that required for juveniles and where costs of their release are minimal. Recommendations describing the practical implementation of processes required for successful stock enhancement are summarised in Table 7.1.

7.1. STOCK ENHANCEMENT

Stock enhancement is considered a potentially valuable tool to support existing traditional fishery management approaches to add value to local populations of *H. rubra* in NSW. This is particularly the case where local populations have become substantially depleted and where a combination of the nature of local depletions, population demography and traditional fisheries management has generally been ineffective in aiding population growth. Throughout this thesis the term 'stock enhancement' has been used to describe the addition of hatchery-reared larvae and/or juveniles to increase the total number of individuals persisting through time, to support a sustainable population, harvestable stock, or both. This generic definition has been used as the research in this thesis was not designed to prescribe a specific objective of a stock enhancement management strategy, be that to support a sustainable ('restocking') or harvestable ('stock enhancement') population, as defined by Bell *et al.* (2005, 2008).

The implementation of a stock enhancement management strategy, and determination of its success, requires a number of integral management structures to be in place and the sound development of specific objectives, agreed to among stakeholders (Cowx 1994, Blankenship & Leber 1995, Cowx 1998, Caddy & Defeo 2003, Tringali *et al.* 2008). However, there are a number of critical requirements that need to be achieved before a stock enhancement program can be implemented and evaluated. Mechanisms for the release of individuals from the hatchery

Table 7.1 Summary of recommendations for the implementation of practical processes required for successful stock enhancement. References refer to sections (*S*) within chapters (C) of this thesis.

Protocol life history stage *(time period post-release)*	**Recommendations**	**Reference**
Handling and release		
larvae[A]	● *handling* ; estimate total number and concentrate to 1 L	C2 - *S 2.2.5*
	● *storage and transport* ; cool storage in thermally insulated container, minimise refrigeration (<24 hr.) and buffer from temperature shock (<10^{o}C)	C2 - *S 2.4.5*
	● *release* ; acclimate to ambient seawater temperature (~20 mins), add to deployment pump, treat with GABA 1 x 10^{-6}M (20 min.), release to niche areas of substratum, targeting CCA-coated substratum with low water flow	C4 - *S 4.4.3* C2 - *S 2.4.5*
juveniles[B]	● *anaesthesia* ; benzocaine at 1.0 ml.L^{-1} for 10 min. (0.5 ml.L^{-1} for 10 min. if seawater temp. >20^{o}C)	C4 - *S 4.2.2.1*
	● *handling* ; estimate total number, distribute among PVC devices (~1 000 device^{-1}), allow recovery from anaesthesia in flow through seawater (12 - 18 hr.)	C4 - *S 4.2.2.2*
	● *storage and transport* ; - short-term transport (<2 hr.); juveniles in devices stacked and separated by cool damp foam - extended transport (2 - 10 hr.); juveniles in devices, stacked and separated by cool damp foam in an insulated container with oxygen, followed by 10 - 48 hr. recovery in seawater tanks	C4 - *S 4.4.2.2*
	● *size at release* (7-15 mm); above this range expected rates of natural mortality decline rapidly and production costs become restrictive	
	● *release* ; juveniles in devices placed securely on substratum by divers and separated by 5 - 10 m, enabling juvenile dispersal throughout location	C4 - *S 4.4.2.3*
Batch-tagging		
larvae	● *larvae* ; immersed in calcein 0.05 g.L^{-1} for 48 hr. (in buffered seawater at 19^{o}C ± 0.5^{o}C)	C3 - *S 3.2.1*
juveniles	● *juveniles* ; off settlement plates (>1.5 mm) and on-grown to release size, fed a commercial abalone diet resulting in distinct blue-green shell colour	C3 - *S 3.2.2*
Monitoring		
larvae[C] *(days to weeks)*	● boulder collection provides greater estimates of short-term survival; venturi-lift has limited application with depth related inefficiencies, under-sampling juveniles >2 mm and limited access to interstitial spaces.	C5 - *S 5.4.1*
juveniles		
short-term *(days to weeks)*	● disturbed boulder sampling; replicates of 0.36m^{2}, *n* >10; this method provides greater and more precise estimates of survival ● a short-term sampling design requires a spatial component to account for dispersal of juveniles from devices within days of release	C5 - *S 5.4.2*
long-term[D] *(months to years)*	● disturbed boulder sampling; replicates of 0.36m^{2}, *n* >10; this method provides greater and more precise estimates of survival ● stratified sampling within 'solid habitat' provides greater estimates of density than disturbed boulder sampling, where habitat has been disturbed ● visual surveys underestimate absolute abundance but provide long-term measures of the abundance of the emergent population (if sampling 'solid habitat')	C5 - *S 5.4.3*

[A] The release of larvae is unlikely to add substantial value to a stock enhancement program as estimates of long-term survival are very low.

[B] Long-term survival of juveniles released in groups of 1 000 was not significantly different from that in groups of 100. Moreover, aggregations of more juveniles may proffer a growth advantage over those released in a more diffuse array (C6 - S 6.3.2.1).

[C] Monitoring the settlement and early survival of released larvae is expensive in terms of field and laboratory time. It is recommended only if it is essential to the program objectives. Alternatively, delay monitoring of released larvae and employ recommended methods to monitor juveniles.

[D] Greater numbers of smaller replicates provide greater levels of precision. However, large sample units may be required where minimum areas of habitat type are required within each replicate. For example, stratified sampling within 'solid' habitat (C5 - *S 5.2.5*). Long-term survival is likely to be greater in more stable habitat types (e.g. solid habitat), this restricts accurate monitoring using methods that disturb the substrata.

to the natural habitat can have a substantial effect on initial and short-term (<60 days) survival (Table 7.1; Chapters 2 and 4). The development of an effective release strategy to transfer hatchery-reared individuals to a release location is essential. It is also essential that individuals released into wild populations be indelibly tagged so that they can be positively identified when recaptured and discriminated from wild conspecifics (Table 7.1; Chapter 3). Further, accurate and representative estimates of the number of individuals surviving through time, and their growth, are critical measures to assess the success of a stock enhancement program (Table 7.1; Chapters 5 and 6). Finally, there is the need to assess the net worth and value of releasing hatchery-reared individuals, i.e. that released individuals do not simply occupy ecological space at the expense of natural recruits and that they provide a comparatively positive return (Chapter 6), as measured against the program's objectives, be they financial and/or biological and compared with alternate fisheries management approaches.

7.2. RELEASE, IDENTIFICATION AND MONITORING

7.2.1. Handling and release

The release of hatchery-reared larvae and juveniles to natural reefs requires a series of logistical steps. Effects of handling, transport and release processes can manifest as an increased rate of mortality prior to release, or as significantly increased mortality within short periods of time (<60 days) thereafter (Tegner & Butler 1985, Schiel & Welden 1987, Schiel 1993, Olla *et al.* 1994, 1998, Purcell *et al.* 2006, Le Vay *et al.* 2007, Lorenzon *et al.* 2007).

Results from the series of controlled laboratory experiments described in Chapter 2 demonstrate that several factors affect the ability of *H. rubra* larvae to successfully settle, survive and grow. Outcomes included the development of a standard protocol for the handling, transport and release of larvae to field locations. Many of the results emphasised the resistance of larvae to the handling and transport processes developed. However, the low rates of settlement over short time periods in the presence of relatively low water flow, emphasise the importance of release methods to control the delivery of larvae to niche habitats that maximise their exposure time to the settlement substrata. This could most readily be achieved through the use of a larval deployment pump (Chapter 4). Combined with recommendations described in Chapter 2, results from the series of experiments investigating the efficiency of delivering competent larvae from this pump supported the development of a standard release protocol for larvae. This protocol was utilised to release large numbers of larvae to replicate locations to investigate its viability to enhance local populations (Chapter 6). The release of tens of thousands of larvae to replicated, enclosed and controlled patches of reef, as similarly reported by Schiel (1992) and Preece *et al.* (1997), indicated significantly greater settlement within enclosures after 24 hours. However, the

application of this process to increase larval settlement on a large scale was considered logistically and economically inefficient.

Results from experiments in Chapter 4 demonstrated that large numbers of hatchery-reared juveniles could be successfully and efficiently released to natural reefs. Further outcomes included the development of standard protocols for the handling and release of juveniles, including that for their anaesthesia and transport. The handling and release of juveniles required additional levels of logistic complexity, compared with that for larvae. The use of a PVC deployment device greatly reduced the physical handling of juveniles throughout this process. The protocol for the extended transport (up to 10 hours) of juveniles resulted in negligible individual mortalities. However, after extended transport, juveniles were often observed to be relatively inactive and were relatively easily detached from the substrata. The treatment of juveniles in seawater tanks for a minimum of 10 hours facilitated their recovery.

Consistent with studies elsewhere (Schiel 1993, McCormick *et al.* 1994, Sweijd *et al.* 1998, Goodsell *et al.* 2006) deployment devices were used in all experiments described in this thesis where juveniles were released. These devices delivered substantial benefits in terms of time and financial savings for the release of juveniles in this thesis, with tens of thousands of juveniles released to locations in <1 diving hour, compared with multiple more hours if hand plantingwas employed (e.g. Schiel 1993). However, in contrast to a number of these other studies, juveniles in these experiments were relatively slow to move off the devices. For example, between 40-60% of juveniles remained on devices after 1 day, although <20% remained after 7-14 days. This was in contrast to that described by Goodsell *et al.* (2006), where they reported rapid (>90%) movement of *H. rubra* (10-12 mm SL) from cylindrical PVC devices, and McCormick *et al.* (1994), where they described the 'usual' movement of between 90-95% of juveniles (25 mm SL) from a similar PVC device after 1-2 days.

7.2.2. Identification - Batch-tagging

Measuring the survival and growth of hatchery-reared and released individuals is an essential requirement to assess the value of a stock enhancement program. To fulfil the objectives of this thesis, it was necessary to reliably and consistently tag all released individuals so that they could be identified when recaptured and distinguished from wild conspecifics up to 2 years later. Batch-tagging enabled recaptured individuals to be identified as whole, live samples; this identification procedure was markedly easier for released juveniles where the shell colour was easily distinguishable with the naked eye, compared with the fluorescent tag of the larval shell that was only visible under specific illumination and magnification (Chapter 3).

The protocols developed from research in this study to batch-tag *H. rubra* larvae are the first to describe a successful method for haliotids and the first to provide evidence of its success from long-term field studies. However, there is the possibility that a proportion of tagged individuals may not have been positively identified when recaptured. As such, estimates of survival were conservative and constituted minimum values for recaptured larvae.

Juvenile abalone were batch-tagged using the blue-green colouration of their shell which was a consequence of their hatchery diet. Moreover, and importantly, the colour of new growth in the shell of released juveniles reverted to a natural red-brown colour shortly after release, leaving the length of each individual at release easily discernable as measured by the length of the batch-tagged 'blue-green' shell in the spire. This change enabled rates of growth to be calculated from recaptured individuals. The batch-tagging protocols employed did not, however, enable estimates of sighting efficiency to be determined, as is possible with unique individual tagging procedures (Shepherd 1990, 1998, Guzman del Proo *et al.* 2004, Dixon *et al.* 2006), nor were they designed to assess population effects of stock enhancement among generations (Rothlisberg & Preston 1992).

7.2.3. Monitoring strategy

Measuring the success of a stock enhancement program requires accurate measures of survival and growth of released individuals (Blankenship & Leber 1995, Munro & Bell 1997, Hilborn 1998, Howell 1998, Caddy & Defeo 2003). Obtaining these data necessitates development of an effective sampling strategy. Development of these strategies for *H. rubra* is complicated by interactions between ontogenetic differences in habitat utilisation, and the conflict between the need to obtain regular, representative samples that do not significantly affect long-term estimates of survival, growth and resource limitations. Finally, the release of juveniles on deployment devices meant their initial distribution was not homogeneous.

A monitoring strategy was developed and tested that enabled quantitative estimates of the abundance of *H. rubra* to be measured at points in time ranging from days and weeks to months and years, after their release to natural reefs as larvae or juveniles (Chapter 5). Different methods used to sample released individuals provided significantly different measures in the accuracy and precision of estimates of density, and consequently abundance, over long periods. Greater numbers of larvae and juveniles were detected using methods that disturbed the habitat. For example, greater numbers of settled larvae and early juveniles were obtained using the collection of boulders than could be achieved using a venturi-lift. However, measuring the settlement and early survival of released larvae is labour intensive and it would be more cost effective to delay monitoring until released individuals are large enough to be monitored using methods to sample larger size classes.

Juvenile abalone released on deployment devices moved up to 5-10 m in 8 days, resulting in a more homogeneous distribution within release locations in short periods of time. This redistribution meant a long-term monitoring strategy could be based on sampling designs that assume even distributions of the target species. It also supported development of a release strategy and definition of release location boundaries and the spatial scale of a long-term monitoring strategy. Further, a common occurrence among release locations was the large-scale disturbance of habitat by rough seas. Stratified sampling within these locations, among strictly defined stratum, revealed more accurate and precise estimates of long-term abundance. This structured sampling approach added substantial value to the long-term monitoring strategy for estimating the survival of released juveniles.

7.3. ASSESSMENT OF STOCK ENHANCEMENT

7.3.1. Survival and growth

Estimating the long-term survival and growth of released individuals in NSW was critical to assessing the value of releasing individuals to a local population (Hilborn 1998, 1999, Bell *et al.* 2005, Lorenzen 2005, Bell *et al.* 2008), and as the basis of the biological component in a bio-economic model to determine the financial viability of a stock enhancement strategy. Long-term estimates of survival and growth from released abalone demonstrate that they can enhance local populations and contribute to the mature population within 2 - 3 years, and the legally harvestable population within 2.5 - 4.5 years. However, estimates of long-term survival and growth varied substantially among locations and through time, in association with factors including habitat loss through rough seas, as similarly described in other studies for released haliotids (Tegner & Butler 1989, Schiel 1993, Roberts *et al.* 2007b) and wild counterparts (Day & Fleming 1992, Shepherd & Breen 1992, Shepherd 1998, Worthington & Andrew 1998, Prince 2004, Saunders & Mayfield 2008). Moreover, that there was no survival of released juveniles at a location within an area of coast where the abundance of wild populations had dramatically declined, that was coincident with reported infections of *P. olseni*, indicates disease may have played a substantial role in the long-term survival of some releases. Consequently, investment in a stock enhancement strategy must balance the risk associated with variation in these important demographic variables, and the nature of the population depletion, against the costs of increased spatial replication or no management response.

This work is the first to directly and quantitatively assess the long-term survival of released abalone larvae. Small proportions of released larvae contributed to local populations after >18 months. However, even with modest increases in survival it is unlikely that the release of larvae could provide a viable, stand alone option for the enhancement of local populations.

However, their release could contribute to a strategy when larval production exceeds requirements and where release methods are effective and cost efficient.

Estimates of the long-term survival of released juveniles in the research described in this thesis were <10%. However, at 25% of locations, long-term survival ranged between 4-10% and was greater than expected for wild conspecifics after >2 years. At the remaining release locations long-term survival was <4% and less than expected for wild *H. rubra* (Chapter 6). Estimates of long-term survival in excess of 4% provided encouraging estimates for the bio-economic assessment of the financial viability of a stock enhancement program. Generally, estimates of long-term survival were among the lower range of similar abalone release programs where hatchery-reared haliotids had been released for >1 year. However, unlike some of the comparative studies, all of the releases done in this study were to areas of un-manipulated natural reef, with some of these releases being to reefs that had experienced substantial declines in extant populations of *H. rubra* associated with infection by *P. olseni*. Rates of growth for juveniles released for >1 year were commonly within a similar range (23-48 $mm.yr^{-1}$), although among higher estimates for wild *H. rubra*. However, the highest mean rate of growth (44 $mm.yr^{-1}$) for juveniles released to multiple locations for >2 years in this study was greater than that recorded among any other known studies for wild *H. rubra.* Notably, the growth of juveniles released in more concentrated groups was significantly greater than that of juveniles released in a diffuse array. These results support a model for density effects on the growth of haliotids in natural habitats that are counter-intuitive (for example, see Huchette *et al.* 2003, Lloyd & Bates 2008) and likely above that for haliotids operating in an artificial environment.

7.3.2. Impact on wild populations of H. rubra

The work reported in this thesis is the first to describe the monitoring of wild and released individuals at multiple release and control locations to assess the long-term impact of releasing individuals on the abundance of wild populations. The impact of releasing individuals on the abundance of the wild population is an important factor in determining the value of a release program (Hilborn 1998, Caddy & Defeo 2003, Hilborn 2004, Bell *et al.* 2005). If released individuals replace, rather than supplement, processes that enable the growth of a wild population, the value of a release program is likely to be negligible (Hilborn 1998, Bell *et al.* 2005).

Results from the controlled release of juveniles among depleted populations at multiple locations indicated that the release of hatchery-reared juveniles did not have a significant negative impact on the abundance of extant populations (Chapter 6). Moreover, natural recruitment and the progression of wild recruits to larger length classes was not significantly different among wild populations where hatchery-reared juveniles were introduced more than 2 years earlier and those

at control locations. That hatchery-reared juveniles can be added to wild populations that have been depleted, and not have a significant effect on natural recruitment, supports the use of stock enhancement as a tool to successfully manage these stocks. Further, these results add support to the arguments put forward for the responsible use of stock enhancement within populations that have been depleted and where depensatory population growth processes operate (Hilborn 2004, Leber 2004, Lorenzen 2005, Bartley & Bell 2008, Lorenzen 2008)

7.3.3. Bio-economic assessment

In this study a long-term (10 year) abalone stock enhancement strategy was described and a deterministic bio-economic model was used to analyse the net present value of this strategy under a range of scenarios, at a comparative rate of return of 10% p.a. (Caddy & Defeo 2003). The use of such a bio-economic analysis is an essential component in the assessment of the financial value of a stock enhancement strategy. This is particularly the case where the objective of the strategy is to support the commercial harvest of released individuals and generate positive economic returns (Hilborn 1998, 1999, Bell *et al.* 2005, Lorenzen 2005, Bell *et al.* 2008). Importantly, the decision to invest in a financially viable stock enhancement program requires the assessment of financial returns within a range of likely economic and biological parameters with comparison against alternative investment opportunities.

The bio-economic analyses done in this thesis used a series of scenarios including biological and economic parameters that ranged between relatively optimistic (fast growth; 6% survival at 2 years; beach price \$AUD 44.kg^{-1}) and relatively pessimistic (slow growth; survival <4% at 2 years; beach price \$AUD 24.kg^{-1}) inputs, from long-term estimates of survival and growth described for *H. rubra* in NSW and from economic data for the commercial fishery. Financial benefits could be gained against an alternate investment returning 10% p.a.. However, these outcomes were generated using scenarios utilising optimistic biological and or economic parameters (Chapter 6). Outcomes generally supported the conclusion that the greatest value of a stock enhancement strategy for *H. rubra* in NSW would be gained where the primary management objective is the rebuilding of depleted natural populations, rather than harvesting released individuals for commercial financial benefit.

7.3.4. Implementation and management

This thesis has provided a mechanism and recommendations to assess the need and implement a stock enhancement management strategy for local populations of *H. rubra* in NSW. A management group consisting of representatives of all stakeholders is required to asses the need for such a strategy, and its implementation requires a complex analysis of multidisciplinary factors applied at the scale of each target population (Caddy & Defeo 2003, Caddy & Agnew

2004, Lorenzen 2005, Bell *et al.* 2008, Lorenzen 2008, Tringali *et al.* 2008). Principal considerations in determining the risk and opportunity costs of implementing a stock enhancement strategy include understanding the demography of the population, the nature of its decline (e.g. overfishing, disease, environmental change), the financial capacity of the fishery and alternate management options that could deliver comparable increases in the value of the resource (e.g. restriction of traditional input and output controls, adult translocation and habitat restoration).

The primary objective of such a strategy requires careful consideration and unambiguous definition. Following from this, production (maintenance of the genetic diversity of the target population) and performance (biological, ecological and financial) targets require explicit description, including methods and timeframes for their assessment and decision rules for continued investment or disinvestment (Chapter 6). Concurrent with these processes is the need for policy development to enable investment decisions to be accurately assessed against the strategy's outcomes (Arnason 2008).

The principal stakeholder responsible for the persistence and provision of functional populations for utilisation by future generations is ultimately the regulatory body accountable for the sustainable management of the resource. In NSW, the initiation of a management process to assess the need and opportunity costs of implementing a stock enhancement strategy for depleted populations lies with state government. However, there are immediate issues limiting a stakeholder assessment of the need, implementation and objectives of a stock enhancement strategy for *H. rubra* in NSW. These include the current capability and capacity of stakeholders to effectively co-manage the fishery under the existing management structure and the limited capacity of stakeholders to invest in such a strategy (Anon 2008b).

7.4. CONCLUSION

The work done in this thesis provides the methodology, baseline targets and recommendations for the implementation of a responsible approach to the stock enhancement of local populations of *H. rubra* in NSW, Australia. The greatest value of a stock enhancement strategy for *H. rubra* in NSW is likely to be gained where the primary management objective is the rebuilding of depleted natural populations, i.e. 'restocking'. Critical to the development and success of this process is the inclusion and collaboration of all stakeholders with considered interests in this resource and fishery. With clearly defined and agreed objectives, and associated measurable targets, the implementation of a successful strategy to enhance local populations of *H. rubra* is possible.

Results from this thesis support the hypothesis that logistically and biologically successful stock enhancement of local wild populations of blacklip abalone can be achieved and critically assessed over long periods of time. Further, outcomes from this work provide logistic protocols for the handling, release, tagging and monitoring of hatchery-reared *H. rubra* larvae and juveniles to natural un-manipulated reefs in NSW (Table 7.1). Baseline targets for successful stock enhancement are described and recommendations are provided to assess the need for, and implementation of a stock enhancement management strategy for local populations of *H. rubra* in NSW, Australia.

7.5. FUTURE RESEARCH

Future research for the responsible development and implementation of a stock enhancement management strategy for *H. rubra* in NSW requires a focus within four important areas. Firstly, production capacity and population integrity are critical to the implementation of a stock enhancement strategy. Production of large numbers of *H. rubra* larvae and juveniles was achieved to support the work described in this thesis. However, more rigorous testing of the effects these innovative and intensive production protocols have on the physiological and behavioural development of hatchery released juveniles would provide greater confidence in the investment in this technology for stock enhancement.

Secondly, low rates of long-term survival of released haliotids has often been attributed to large-scale habitat disturbance. This was also the case in this thesis. Therefore, despite the limited application of generalisations regarding the success of releasing individuals, it is likely that significantly higher average rates of long-term survival could be achieved through the targeting of areas of reef displaying more stable habitat. Comparable, or increased rates of long-term survival among more stable habitats would require appropriate testing before its broad-scale application. Further, investigation into the response of local populations to stock enhancement, after having been depleted by processes such as disease or recruitment over-fishing, would provide greater direction in the application of this strategy for management.

Thirdly, bio-economic analysis of a simulated, long-term stock enhancement strategy showed that positive financial returns can be made against an alternate investment opportunity returning 10% p.a., where rates of survival and growth were above the average measures obtained from long-term experiments. More detailed bio-economic analyses, including greater determination of risk could be achieved through the application of stochasticity in some parameters of the model.

Finally, critical issues for the responsible implementation of a large-scale stock enhancement program include maintaining the genetic diversity of wild populations, through appropriate brood-stock and breeding management protocols and the long-term functionality of the broader

ecological system. A socially, politically and scientifically acceptable level of difference in the genetic diversity of the original population against a future, enhanced population requires definition and testing. This would facilitate the making of acceptable, responsible management decisions about the termination or continuation of a program. Similarly, measures of the impact of releasing *H. rubra* on competitors, predators and/or other ecosystem measures in which an enhanced *H. rubra* population would function requires scientifically rigorous, long-term assessment to support the implementation and continuation of a responsible stock enhancement strategy.

8. REFERENCES

Andrew N, Worthington D, Brett P, Bentley N, Chick R, Blount C (1998) Interactions between the abalone fishery and sea urchins in New South Wales. Final Report Project 93/102., Fisheries Research and Development Corporation, Canberra.

Andrew N, Worthington D, Chick R, Brett P, Avery R, Hayes E (1996) An assessment of the NSW abalone, sea urchin and turban fishery for 1995, New South Wales Fisheries Research Institute, Sydney.

Andrew NL, Mapstone BD (1987) Sampling and the description of spatial pattern in marine ecology. Annual Review of Oceanography and Marine Biology 25:39-90.

Andrew NL, Underwood AJ (1992) Associations and abundance of sea-urchins and abalone on shallow subtidal reefs in southern New South Wales. Australian Journal of Marine and Freshwater Research 43:1547-1559.

Andrew NL, Worthington D, Brett P (1997) Size-structure and growth of individuals suggest high exploitation rates in the fishery for blacklip abalone, *Haliotis rubra*, in New South Wales, Australia. Molluscan Research 18:275-287.

Andrewartha HG, Birch LC (1984) The ecological web: more on the distribution and abundance of animals. University of Chicago Press

Anguiano-Beltran C, Searcy-Bernal R (2007) Effects of antibiotics on the concentration of bacteria in biofilms and on the growth of *Haliotis rufescens* postlarvae. Journal of Shellfish Research 26:795-799.

Anon (2005a) Assessment of the New South Wales Abalone Fishery, Department of the Environment and Heritage, Canberra, Australia.

Anon (2005b) Environmental Impact Statement on the Abalone Fishery in NSW. Public consultation document, The Ecology Lab Pty Ltd, Sydney, NSW, Australia.

Anon (2005c) Total allowable catch committee. Abalone fishery report and determination for 2005-6, New South Wales Department of Primary Industries - Fishing and aquaculture

Anon (2008a) Abalone Management Advisory Committee Meeting 18 April 2008, New South Wales Department of Primary Industries - Fishing and aquaculture

Anon (2008b) Total allowable catch committee. Report and determination for 2008/09. Abalone Fishery, New South Wales Department of Primary Industries - Fishing and aquaculture

Appleford P, Doroudi M, Hardy-Smith P (2008) Abalone viral ganglioneuritis in south eastern Australia - A wake up call. Journal of Shellfish Research 27:986-986.

Aquilina B, Roberts R (2000) A method for inducing muscle relaxation in the abalone, *Haliotis iris.* Aquaculture 190:403-408.

Arnason R (2008) On the economics of releasing cultured fish into the aquatic environment. Reviews in Fisheries Science 16:135-145.

Ault JS, DeMartini JD (1987) Movement and dispersion of red abalone, *Haliotis rufescens*, in Northern California. California Fish and Game 73:196-213.

Babcock RC, Keesing JK (1999) Fertilization biology of the abalone *Haliotis laevigata*: laboratory and field studies. Canadian Journal of Fisheries and Aquatic Sciences 56:1668-1678.

Baloun AJ, Morse DE (1984) Ionic control of settlement and metamorphosis in larval *Haliotis rufescens* (Gastropoda). Biological Bulletin 167:124-138.

Barrett NS, Buxton CD, Edgar GJ (2009) Changes in invertebrate and macroalgal populations in Tasmanian marine reserves in the decade following protection. Journal of Experimental Marine Biology and Ecology 370:104-119.

Barrett NS, Edgar GJ, Buxton CD, Haddon M (2007) Changes in fish assemblages following 10 years of protection in Tasmanian marine protected areas. Journal of Experimental Marine Biology and Ecology 345:141-157.

Barrowman NJ, Myers RA (1996) Estimating tag-shedding rates for experiments with multiple tag types. Biometrics 52:1410-1416.

Bartley DM, Bell JD (2008) Restocking, stock enhancement, and sea ranching: Arenas of progress. Reviews in Fisheries Science 16:357-365.

Bartley DM, Bondad-Reantaso MG, Subasinghe RP (2006) A risk analysis framework for aquatic animal health management in marine stock enhancement programmes. Fisheries Research 80:28-36.

Bashey F (2004) A comparison of the suitability of alizarin red S and calcein for inducing a nonlethally detectable mark in juvenile guppies. Transactions of the American Fisheries Society 133:1516-1523.

Beaudry CG (1983) Survival and growth of the larvae of *Haliotis kamtschatkana* Jonas at different temperatures. J Shellfish Res 3:109-110.

Bell JD, Bartley DM, Lorenzen K, Loneragan NR (2006) Restocking and stock enhancement of coastal fisheries: Potential, problems and progress. Fisheries Research 80:1-8.

Bell JD, Leber KM, Blankenship HL, Loneragan NR, Masuda R (2008) A new era for restocking, stock enhancement and sea ranching of coastal fisheries resources. Reviews in Fisheries Science 16:1-9.

Bell JD, Rothlisberg PC, Munro JL, Loneragan NR, Nash WJ, Ward RD, Andrew NL (2005) Advances in Marine Biology. Restocking and Stock Enhancement of Marine Invertebrate Fisheries. Elsevier Academic Press Inc, San Diego.

Berka R (1986) The transport of live fish. A review. EIFAC (European Inland Fisheries Advisory Commission) Technical Paper:1-52.

Berryman AA (2002) Population: a central concept for ecology? Oikos 97:439-442.

Blankenship HL, Leber KM (1995) A responsible approach to marine stock enhancement. American Fisheries Society Symposium 15:167-175.

Branch GM, Clark BM (2006) Fish stocks and their management: The changing face of fisheries in South Africa. Marine Policy 30:3-17.

Breen P, Kim S (2003) The 2003 stock assessment of paua (*Haliotis iris*) in PAU7. New Zealand Fisheries Assessment Report 2003/35.

Breen PA (1986) Management of the British Columbia fishery for northern abalone (*Haliotis kamtschatkana*). Canadian Special Publication of Fisheries and Aquatic Sciences 92:300-312.

Brooks RC, Heidinger RC, Kohler CC (1994) Mass-marking otoliths of larval and juvenile walleyes by immersion in oxytetracycline, calcein, or calcein blue. North American Journal of Fisheries Management 14:143-150.

Bumguardner BW, King TL (1996) Toxicity of oxytetracycline and calcein to juvenile striped bass. Transactions of the American Fisheries Society 125:143-145.

Butman CA, Grassle JP, Webb CM (1988) Substrate choices made by marine larvae settling in still water and in a flume flow. Nature 333:771-773.

Caddy JF, Agnew DJ (2004) An overview of recent global experience with recovery plans for depleted marine resources and suggested guidelines for recovery planning. Reviews in Fish Biology and Fisheries 14:43-112.

Caddy JF, Defeo O (2003) Enhancing or restoring the productivity of natural populations of shellfish and other marine invertebrate resources. FAO Fisheries Technical Paper. No. 448, Food and Agriculture Organization of the United Nations, Rome, Italy.

Caley MJ, Carr MH, Hixon MA, Hughes TP, Jones GP, Menge BA (1996) Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics 27:477-500.

Camus PA, Lima M (2002) Populations, metapopulations, and the open-closed dilemma: the conflict between operational and natural population concepts. Oikos 97:433-437.

Catalano MJ, Chipps SR, Bouchard MA, Wahl DH (2001) Evaluation of injectable fluorescent tags for marking centrarchid fishes: retention rate and effects on vulnerability to predation. North American Journal of Fisheries Management 21:911-917.

Chacon O, Viana MT, Farias A, Vazquez C, Garcia-Esquivel Z (2003) Circadian metabolic rate and short-term response of juvenile green abalone (*Haliotis fulgens* Philippi) to three anaesthetics. Journal of Shellfish Research 22:415-421.

Chick RC, Mayfield S, Turich N, Foureur B (2006) Western Zone Abalone (*Haliotis laevigata & H. rubra*) Fishery 1. Region A. Fisheries assessment report to PIRSA Fisheries. SARDI Publication No. RD05/0017-3, South Australian Research and Development Institute (Aquatic Sciences), Adelaide

Chiu Liao I (1999) How can stock enhancement and sea ranching help sustain and increase coastal fisheries? In: Howell BR, Moksness E, Svåsand T (eds) Stock Enhancement and Sea Ranching. Blackwell Science Ltd, Oxford, p 132-149.

Christensen V, Guénette S, Heymans JJ, Walters CJ, Watson R, Zeller D, Pauly D (2003) Hundred-year decline of North Atlantic predatory fishes. Fish and Fisheries 4:1-24.

Collin R, Voltzow J (1998) Initiation, calcification, and form of larval "archaeogastropod" shells. Journal of Morphology 235:77-89.

Connell JH (1985) The consequences of variation in initial settlement vs post-settlement mortality in rocky intertidal communities. Journal of Experimental Marine Biology and Ecology 93:11-45.

Conover DO, Travis J, Coleman FC (2000) Essential fish habitat and marine reserves: an introduction to the Second Mote Symposium in Fisheries Ecology. Bulletin of Marine Science 66:527-534.

Cook PA, Sweijd N (1999) The Potential for Abalone Ranching and Enhancement in South Africa. In: Howell BR, Moksness E, Svåsand T (eds) Stock Enhancement and Sea Ranching Papers from the First International Symposium on Stock Enhancement and Sea Ranching, Bergen, Norway, 8-11 September 1997. Fishing News Books Ltd., Oxford UK, p 453-467.

Cowx IG (1994) Stocking strategies. Fisheries Management and Ecology 1:15-30.

Cowx IG (1998) Stocking strategies: issues and options for future enhancement programmes. In: Cowx IG (ed) Stocking and Introduction of Fish. Blackwell Science Ltd, Oxford, UK, p 3-13.

Crowe TP, Lee CL, McGuinness KA, Amos MJ, Dangeubun J, Dwiono SAP, Makatipu PC, Manuputty J, N'Guyen F, Pakoa K, Tetelepta J (2002) Experimental evaluation of the use of hatchery-reared juveniles to enhance stocks of the topshell *Trochus niloticus* in Australia, Indonesia and Vanuatu. Aquaculture 206:175-197.

Croxall JP, Nicol S (2004) Management of Southern Ocean fisheries: global forces and future sustainability. Antarctic Science 16:569-584.

Daly T (2004) Summary of Proceedings from the Perkinsus Workshop held at the Cronulla Fisheries Centre on 3 September 2003. Fisheries Research Report Series, Vol 10. New South Wales Fisheries, Sydney, Australia, p 36.

Daume S, Brand-Gardner S, Woelkerling WJ (1999) Preferential settlement of abalone larvae: diatom films vs. non geniculate coralline red algae. Aquaculture 174:243-254.

Daume S, Huchette S, Ryan S, Day RW (2004) Nursery culture of *Haliotis rubra*: the effect of cultured algae and larval density on settlement and juvenile production. Aquaculture 236:221-239.

Daume S, Krsinich A, Farrel S, Gervis M (2000) Settlement and early growth and survival of *Haliotis rubra* in response to different algal species. Journal of Applied Phycology 12:479-488.

Daume S, Ryan S (2004) Fatty acid composition of eggs derived from conditioned and wild caught greenlip abalone broodstock (*Haliotis laevigata*). Journal of Shellfish Research 23:967-974.

Davis GE (1995) Recruitment of juvenile abalone (*Haliotis* spp.) measured in artificial habitats. Marine and Freshwater Research 46:549-554.

Day E, Branch GM (2000a) Evidence for a positive relationship between juvenile abalone *Haliotis midae* and the sea urchin *Parechinus angulosus* in the south-western Cape, South Africa. South African Journal of Marine Science 22:145-156.

Day R, Branch GM (2000b) Relationships between recruits of the abalone *Haliotis midae*, encrusting corallines and the sea urchin *Parechinus angulosus*. South African Journal of Marine Science 22:137-144.

Day RW, Fleming AE (1992) The determinants and measurement of abalone growth. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 141-168.

Day RW, Williams MC, Hawkes GP (1995) A comparison of fluorochromes for marking abalone shells. Marine and Freshwater Research 46:599-605.

Dayton PK (1998) Ecology - Reversal of the burden of proof in fisheries management. Science 279:821-822.

de Waal S, Cook P (2001) Quantifying the physical and biological attributes of successful ocean seeding sites for farm-reared juvenile abalone (*Haliotis midae*). Journal of Shellfish Research 20:857-861.

de Waal S, Sweijd N, Godfrey B, Britz P, Cook P (1999) Abalone ranching in South Africa: Hope for sustainable abalone production? Journal of Shellfish Research 18:714.

de Waal SWP (2005) Boulders or urchins? Selecting seeding sites for juvenile *Haliotis midae* along the Namaqualand coast of South Africa. African Journal of Marine Science 27:501-504.

de Waal SWP, Branch GM, Navarro R (2003) Interpreting evidence of dispersal by *Haliotis midae* juveniles seeded in the wild. Aquaculture 221:299-310.

Dixon CD, Day RW (2004) Growth responses in emergent greenlip abalone to density reductions and translocations. Journal of Shellfish Research 23:1223-1228.

Dixon CD, Day RW, Huchette SMH, Shepherd SA (2006) Successful seeding of hatchery-produced juvenile greenlip abalone to restore wild stocks. Fisheries Research 78:179-185.

Dixon CD, Gorfine HK, Officer RA, Sporcic M (1998) Dispersal of tagged blacklip abalone, *Haliotis rubra*: implications for stock assessment. Journal of Shellfish Research 17:881-887.

Doherty PJ (1999) Recruitment limitation is the theoretical basis for stock enhancement in marine populations. In: Howell BR, Moksness E, Svåsand T (eds) Stock Enhancement and Sea Ranching. Blackwell Science Ltd, Oxford, p 9-21.

Drummond SP, Connell SD (2005) Quantifying percentage cover of subtidal organisms on rocky coasts: a comparison of the costs and benefits of standard methods. Marine and Freshwater Research 56:865-876.

Dulvy NK, Sadovy Y, Reynolds JD (2003) Extinction vulnerability in marine populations. Fish and Fisheries 4:25-64.

Ebert EE, Houk JL (1984) Elements and innovations in the cultivation of red abalone *Haliotis rufescens*. Aquaculture 39:375-392.

Ebert TB, Ebert DA (1991) Experimental abalone population enhancement project: Fitzgerald Marine Reserve, San Mateo County, California. Final Report, Department of Environmental Management, Parks and Recreation Division, County Government Center, Redwood City, California, USA.

Ebert TB, Ebert EE (1988) An innovative technique for seeding abalone and preliminary results of laboratory and field trials. California Fish and Game 74:68-81.

Eckman JE (1996) Closing the larval loop: Linking larval ecology to the population dynamics of marine benthic invertebrates. Journal of Experimental Marine Biology and Ecology 200:207-237.

Edwards S, Johns D, Burke CM (2000) Recovery and Growth Effects of Anaesthetics on Greenlip and Blacklip Abalone. In: Burke CM, Harris JO, Hindrum SM, Edwards SJ, Maguire GB (eds) Environmental Requirements of Abalone Final Report Project 97/323. Fisheries Research and Development Corporation, Canberra, p 116-128.

Emmett B, Jamieson GS (1988) Transplant of abalone in Barkley Sound, British Columbia. Journal of Shellfish Research 7:117.

Emmett B, Jamieson GS (1989) An experimental transplant of northern abalone, *Haliotis kamtschatkana*, in Barkley sound, British Columbia. Fishery Bulletin 87:95-104.

Estudillo CB, Duray MN (2003) Transport of hatchery-reared and wild grouper larvae, *Epinephelus* sp. Aquaculture 219:279-290.

FAO (1996) Precautionary approach to capture fisheries and species introductions. FAO Fisheries Technical Paper 350, Part 1, Food and Agriculture Organization of the United Nations, Rome.

FAO (2010) Fisheries and Aquaculture Department. Global Production Statistics (online query) http://www.fao.org/fishery/statistics/global-production/en. Food and Agriculture Organization of the United Nations, Rome.

Foster KR, Vecchia P, Rapacholi MH (2000) Science and the precautionary principle. Science 279:821-822.

Fowler CW, Baker JD (1991) A review of animal population dynamics at extremely reduced population levels. Report of the International Whaling Commission 41:545-554.

Gallardo WG, Bautista-Teruel MN, Fermin AC, Marte CL (2003) Shell marking by artificial feeding of the tropical abalone *Haliotis asinina* Linne juveniles for sea ranching and stock enhancement. Aquaculture Research 34:839-842.

Gardmark A, Enberg K, Ripa J, Laakso J, Kaitala V (2003) The ecology of recovery. Annales Zoologici Fennici 40:131-144.

Gibson PT, Worthington DG, Blount C, Andrew NL (2002) Incidental damage of blacklip abalone (*Haliotis rubra*) by commercial divers in New South Wales, Australia. Journal of Shellfish Research 21:787-792.

Glazer R (2005) A model for decoupling emigration from mortality in recapture surveys of slow-moving benthic marine gastropods. Journal of Shellfish Research 24:387-392.

Goggin CL, Lester RJG (1995) *Perkinsus*, a protistan parasite of abalone in Australia: a review. Marine and Freshwater Research 46:639-646.

Goodsell PJ, Underwood AJ, Chapman MG, Heasman MP (2006) Seeding small numbers of cultured black-lip abalone (*Haliotis rubra* Leach) to match natural densities of wild populations. Marine and Freshwater Research 57:747-756.

Gordon HR, Cook PA (2001) World abalone supply, markets and pricing: historical, current and future. Journal of Shellfish Research 20 (2):567-570.

Gordon HR, Cook PA (2004) World abalone fisheries and aquaculture update: supply and market dynamics. Journal of Shellfish Research 23 (4):935-939.

Gorfine HK, Forbes DA, Gason AS (1998) A comparison of two underwater census methods for estimating the abundance of the commercially important blacklip abalone, *Haliotis rubra*. Fishery Bulletin 96:438-450.

Gutierrez-Gonzalez JL, Perez-Enriquez R (2005) A genetic evaluation of stock enhancement of blue abalone *Haliotis fulgens* in Baja California, Mexico. Aquaculture 247:233-242.

Guzman del Proo SA (1992) A review of the biology of abalone and its fishery in Mexico. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 341-360.

Guzman del Proo SA, Carrillo-Laguna J, Belmar-Perez J, Carreon-Palau L, Castro A (2004) Transplanting of wild and cultivated juveniles of green abalone (*Haliotis fulgens* Philippi 1845): growth and survival. Journal of Shellfish Research 23:855-860.

Haaker PL, Parker DO, Togstad H (1992) Mass mortality and withering syndrome in black abalone, *Haliotis cracherodii*, in California. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the world: biology, fisheries and culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 214-224.

Hahn KO (1989a) Biotic and abiotic factors affecting the culture of abalone. In: Hahn KO (ed) Handbook of Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, Florida, p 129–134.

Hahn KO (1989b) Larval development of abalone. In: Hahn KO (ed) Handbook of Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, Florida, p 71-99.

Hahn KO (1989c) Nutrition and growth in abalone. In: Hahn KO (ed) Handbook of Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, Florida, p 135-156.

Hamasaki K, Kitada S (2008) The enhancement of abalone stocks: lessons from Japanese case studies. Fish and Fisheries 9:243-260.

Hamilton Al (1969) A method of separating invertebrates from sediments using longwave ultraviolet light and fluorescent dyes. Journal of the Fisheries Research Board of Canada 26:1667-1672.

Hart AM, Fabris FP, Daume S (2007) Stock enhancement of *Haliotis laevigata* in Western Australia - a preliminary assessment. Report No. 166, Department of Fisheries, Western Australia

Hart AM, Gorfine HK, Callan MP (1997) Abundance estimation of blacklip abalone (*Haliotis rubra*) I. An analysis of diver-survey methods used for large-scale monitoring. Fisheries Research 29:159-169.

Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, Hofmann EE, Lipp EK, Osterhaus ADME, Overstreet RM, Porter JW, Smith GW, Vasta GR (1999) Emerging marine diseases- climate links and anthropogenic factors. Science 285:1505-1510.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfield RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296:2158-2162.

Heasman M, Chick R, Savva N, Worthington D, Brand C, Gibson P, Diemar J (2004) Enhancement of populations of abalone in NSW using hatchery-produced seed. FRDC No. 1998/219. Report No. 62, New South Wales Fisheries, Sydney, Australia.

Heasman MP (2006) In pursuit of cost-effective fisheries enhancement of New South Wales blacklip abalone, *Haliotis rubra* (Leach) fishery. Journal of Shellfish Research 25:211-224.

Hilborn R (1998) The economic performance of marine stock enhancement projects. Bulletin of Marine Science 62 (2):661-674.

Hilborn R (1999) Confessions of a reformed hatchery basher. Fisheries 24:30-31.

Hilborn R (2003) The state of the art in stock assessment: where we are and where we are going. Scientia Marina 67:15-20.

Hilborn R (2004) Population management in stock enhancement and sea ranching. In: Leber KM, Kitada S, Blankenship HL, Svåsand T (eds) Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Science Ltd, Oxford, UK, p 201-210.

Hilborn R, Branch TA, Ernst B, Magnussson A, Minte-Vera CV, Scheuerell MD, Valero JL (2003) State of the world's fisheries. Annual Review of Environment and Resources 28:359-399.

Hilborn R, Walters CJ (1992) Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York, USA.

Hines AH, Pearse JS (1982) Abalones, shells, and sea otters: dynamics of prey populations in central California. Ecology 63:1547-1560.

Hobday AJ, Tegner MJ, Haaker PL (2001) Over-exploitation of a broadcast spawning marine invertebrate: decline of the white abalone. Reviews in Fish Biology and Fisheries 10:493-514.

Hooker SH (1988) The demography of paua (*Haliotis iris*), with special reference to juveniles. Masters thesis, University of Auckland.

Howell B (1998) Marine Fisheries. In: Cowx IG (ed) Stocking and Introduction of Fish. Blackwell Science Ltd, Oxford, UK, p 289-293.

Huchette SMH, Koh CS, Day RW (2003) Growth of juvenile blacklip abalone (*Haliotis rubra*) in aquaculture tanks: effects of density and ammonia. Aquaculture 219:457-470.

Huchette SMH, Soulard JP, Koh CS, Day RW (2004) Maternal variability in the blacklip abalone, *Haliotis rubra* Leach (Mollusca: Gastropoda): effect of egg size on fertilisation success. Aquaculture 231:181-195.

Huggett MJ, de Nys R, Williamson JE, Heasman M, Steinberg PD (2005) Settlement of larval blacklip abalone, *Haliotis rubra*, in response to green and red macroalgae. Marine Biology 147:1155-1163.

Hutchings JA, Walters CJ, Haedrich RL (1997) Is scientific enquiry incompatible with government information control? Canadian Journal of Fisheries and Aquatic Sciences 54:1198-1210.

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629-637.

James DS, Day RW, Shepherd SA (2007) Experimental abalone ranching on artificial reef in Port Phillip Bay, Victoria. Journal of Shellfish Research 26:687-695.

Kawamura T (1996) The role of benthic diatoms in the early life stages of the Japanese abalone (*Haliotis discus hannai*). In: Watanabe Y, Yamashita Y, Oozeki Y (eds) Survival Strategies in Early Life Stages of Marine Resources. A.A. Balkema, Rotterdam, p 355-367.

Kawamura T (2007) Selective settlement of abalone larvae and its ecological implications. Sessile Organisms 24:95-102.

Kawamura T, Roberts RD, Takami H (1998) A review of the feeding and growth of post-larval abalone. Journal of Shellfish Research 17:615-625.

Keesing JK, Grovejones R, Tagg P (1995) Measuring settlement intensity of abalone- results of a pilot study. Marine and Freshwater Research 46:539-543.

Kellison GT, Eggleston DB, Burke JS (2000) Comparative behaviour and survival of hatchery-reared versus wild summer flounder (*Paralichthys dentatus*). Canadian Journal of Fisheries and Aquatic Sciences 57:1870-1877.

Kemp R (2001) Effect of diet constituents on abalone (*Haliotis laevigata*) intestinal structure and function Proceedings of the 8th Annual Abalone Aquaculture Workshop, Fremantle, Western Australia, p 153-177.

Keough MJ, Downes BJ (1982) Recruitment of marine invertebrates - the role of active larval choices and early mortality. Oecologia 54:348-352.

Kitada S (1999) Effectiveness of Japan's stock enhancement programmes: current perspectives. In: Howell BR, Moksness E, Svåsand T (eds) Stock Enhancement and Sea Ranching. Blackwell Science Ltd, Oxford, p 103-131.

Kiyomoto S, Watanabe S, Suenaga T, Yamanaka H, Fujii A, Koshiishi Y, Horii T (2006) Utility of artificial settlement collector using crustose coralline algae for sampling abalone larvae in southwestern Japan. Bulletin of Fisheries Research Agency 5:91-96.

Kiyomoto S, Yamasaki M (1999) Size dependent changes in habitat, distribution and food habit of juvenile disc abalone *Haliotis discus discus* on the coast of Nagasaki Prefecture, southwest Japan. Bulletin of Tohoku National Fisheries Research Institute 62:71-81.

Kojima H (1981) Mortality of young Japanese black abalone *Haliotis discus* after transplantation. Bulletin of the Japan Society of Scientific Fisheries 47:151-159.

Kojima H (1995) Evaluation of abalone stock enhancement through the release of hatchery-reared seeds. Marine and Freshwater Research 46:689-695.

Kritzer JP, Sale PF (2004) Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Fish and Fisheries 5:131-140.

Kriwoken LK, Rose G, Bache S (2001) Applying the precautionary principle to Australian marine and coastal environments. Report No. 26, Cooperative Research Centre for the Antarctic and the Southern Ocean, Hobart, Australia.

Lackey RT (2003) Pacific Northwest Salmon: Forecasting their status in 2100. Reviews in Fisheries Science 11:35-88.

Larkin PA (1996) Concepts and issues in marine ecosystem management. Reviews in Fish Biology and Fisheries 6:139-164.

Lauck T, Clark CW, Mangel M, Munro GR (1998) Implementing the precautionary principle in fisheries management through marine reserves. Ecological Applications 8:S72-S78.

Le Vay L, Carvalho GR, Quinitio ET, Lebata JH, Ut VN, Fushimi H (2007) Quality of hatchery-reared juveniles for marine fisheries stock enhancement. Aquaculture 268:169-180.

Leber KM (1999) Rationale for an experimental approach to stock enhancement. In: Howell BR, Moksness E, Svåsand T (eds) Stock Enhancement and Sea Ranching. Blackwell Science Ltd, Oxford, p 63-75.

Leber KM (2004) Marine stock enhancement in the USA: Status, trends and needs. In: Leber KM, Kitada S, Blankenship HL, Svåsand T (eds) Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Science Ltd, Oxford, UK, p 11-24.

Leber KM, Brennan NP, Arce SM (1995) Marine enhancement with striped mullet: are hatchery releases replenishing or displacing wild stocks? American Fisheries Society Symposium 15:376-387.

Leighton DL (1961) Observations on the effects of diet on shell coloration in the red abalone *Haliotis rufescens* Swainson. Veliger 4:29-32.

Leighton DL (1972) Laboratory observations on the early growth of the abalone, *Haliotis sorenseni*, and the effect of temperature on larval development and settling success. Fishery Bulletin 70 (2):373-381.

Leighton DL (1985) Early growth of the green abalone in hatchery and field. In: Mitchell C (ed) Proceedings of the Joint International Scientific Diving Symposium. American Academy of Underwater Sciences, La Jolla, CA, p 235-248.

Leighton DL (1989) Abalone (Genus: *Haliotis*) mariculture on the North American Pacific coast. Fishery Bulletin 87 (3):689-702.

Leips J, Baril CT, Rodd FH, Reznick DN, Bashey F, Visser GJ, Travis J (2001) The suitability of calcein to mark poeciliid fish and a new method of detection. Transactions of the American Fisheries Society 130:501-507.

Levin LA (1990) A review of methods for labeling and tracking marine invertebrate larvae. Ophelia 32:115-144.

Lloyd MJ, Bates AE (2008) Influence of density-dependent food consumption, foraging and stacking behaviour on the growth rate of the Northern abalone, *Haliotis kamtschatkana*. Aquaculture 277:24-29.

Lorenzen K (2005) Population dynamics and potential of fisheries stock enhancement: practical theory for assessment and policy analysis. Philosophical Transactions of the Royal Society B- Biological Sciences 360:171-189.

Lorenzen K (2008) Understanding and managing enhancement fisheries systems. Reviews in Fisheries Science 16:10-23.

Lorenzon S, Giulianini PG, Martinis M, Ferrero EA (2007) Stress effect of different temperatures and air exposure during transport on physiological profiles in the American lobster *Homarus americanus*. Comparative Biochemistry and Physiology A- Molecular & Integrative Physiology 147:94-102.

Lubchenco J, Palumbi SR, Gaines SD, Andelman S (2003) Plugging a hole in the ocean: the emerging science of marine reserves. Ecological Applications 13:S3-S7.

Malham SK, Lacoste A, Gelebart F, Cueff A, Poulet SA (2003) Evidence for a direct link between stress and immunity in the mollusc *Haliotis tuberculata.* Journal of Experimental Zoology Part A- Comparative Experimental Biology 295A:136-144.

Manahan DT, Jaeckle WB (1992) Implications of Dissolved Organic Material in Seawater for the Energetics of Abalone Larvae *Haliotis rufescens*: A Review. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK

Masuda R, Tsukamoto K (1998) Stock enhancement in Japan: review and perspective. Bulletin of Marine Science 62 (2):337-358.

Masuda R, Ziemann DA (2003) Vulnerability of Pacific threadfin juveniles to predation by bluefin trevally and hammerhead shark: size dependent mortality and handling stress. Aquaculture 217:249-257.

McCormick TB, Herbinson K, Mill TS, Altick J (1994) A review of abalone seeding, possible significance and a new seeding device. Bulletin of Marine Science 55:680-693.

McShane P, Naylor R (1996) Variation in spawning and recruitment of *Haliotis iris* (Mollusca: Gastropoda). New Zealand Journal of Marine and Freshwater Research 30:325-332.

McShane PE (1991) Density-dependent mortality of recruits of the abalone *Haliotis rubra* (Mollusca: Gastropoda). Marine Biology 110:385-389.

McShane PE (1992) Early life history of abalone: a review. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 120-138.

McShane PE (1994) Estimating the abundance of abalone (*Haliotis* spp.) stocks - examples from Victoria and southern New Zealand. Fisheries Research 19:379-394.

McShane PE (1995) Recruitment variation in abalone: its importance to fisheries management. Marine and Freshwater Research 46:555-570.

McShane PE, Black KP, Smith MG (1988a) Recruitment processes in *Haliotis rubra* (Mollusca: Gastropoda) and regional hydrodynamics in south-eastern Australia imply localized dispersal of larvae. Journal of Experimental Marine Biology and Ecology 124:175-203.

McShane PE, Naylor JR (1995a) Density independent growth of *Haliotis iris* Martyn (Mollusca: Gastropoda). Journal of Experimental Marine Biology and Ecology 190:51-60.

McShane PE, Naylor JR (1995b) Depth can effect post settlement survival of *Haliotis iris* (Mollusca: Gastropoda). Journal of Experimental Marine Biology and Ecology 187:1-12.

McShane PE, Smith MG (1988) Measuring abundance of juvenile abalone, *Haliotis rubra* Leach (Gastropoda: Haliotidae); comparison of a novel method with two other methods. Australian Journal of Marine and Freshwater Research 39:331-336.

McShane PE, Smith MG (1991) Recruitment variation in sympatric populations of *Haliotis rubra* (Mollusca: Gastropoda) in southeast Australian waters. Marine Ecology Progress Series 73:203-210.

McShane PE, Smith MG (1992) Shell growth checks are unreliable indicators of age of the abalone *Haliotis rubra* (Mollusca: Gastropoda). Australian Journal Marine Freshwater Research 42:1215-1219.

McShane PE, Smith MG, Beinssen KHH (1988b) Growth and morphometry in abalone (*Haliotis rubra* Leach) from Victoria. Australian Journal Marine Freshwater Research 39:161-166.

Miller JM, Walters CJ (2004) Experimental ecological tests within stocked marine fish. In: Leber KM, Kitada S, Blankenship HL, Svåsand T (eds) Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Science Ltd, Oxford, UK, p 142-152.

Miner CM, Altstatt JM, Raimondi PT, Minchinton TE (2006) Recruitment failure and shifts in community structure following mass mortality limit recovery prospects of black abalone. Marine Ecology Progress Series 327:107-117.

Momma H, Kobayashi T, Kato Y, Sasaki T, Sakamoto T, Murata H (1980) Results of the abalone culture in shallow water of a rocky coast. I. Survival of laboratory cultured seed (*Haliotis discus hannai*) when transplanted into concrete-beam cribs filled with rocks. The Aquaculture 28:59-65.

Moran AL (2000) Calcein as a marker in experimental studies newly-hatched gastropods. Marine Biology 137:893-898.

Moran AL, Marko PB (2005) A simple technique for physical marking of larvae of marine bivalves. Journal of Shellfish Research 24:567-571.

Morse ANC, Morse DE (1984) Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surfaces of crustose red algae. Journal of Experimental Marine Biology and Ecology 75:191-125.

Morse DE (1990) Recent progress in larval settlement and metamorphosis: closing the gaps between molecular biology and ecology. Bulletin of Marine Science 46:465-483.

Morse DE (1992) Molecular Mechanisms Controlling Metamorphosis and Recruitment in Abalone. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 107-119.

Morse DE, Hooker N, Duncan H, Jensen L (1979) gama-Aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. Science 204:407-410.

Moss GA, Tong LJ (1992a) Effect of stage of larval development on the settlement of the abalone, *Haliotis iris.* New Zealand Journal of Marine and Freshwater Research 26:69-73.

Moss GA, Tong LJ (1992b) Techniques for enhancing larval settlement of the abalone, *Haliotis iris*, on artificial surfaces. New Zealand Journal of Marine and Freshwater Research 26:75-79.

Mundy C, Karlov T, Haddon M (2006) Linking fishery-dependent and fishery-independent assessments of abalone fisheries. Final report to the Australian Fisheries Research and Development Corporation. FRDC Project No. 2001/074, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania

Munro JL, Bell JD (1997) Enhancement of marine fisheries resources. Reviews in Fisheries Science 5:185-222.

Myers RA, Barrowman NJ, Hutchings JA, Rosenberg AA (1995) Population-dynamics of exploited fish stocks at low population-levels. Science 269:1106-1108.

Myers RA, Hutchings JA, Barrowman NJ (1997) Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecological Applications 7:91-106.

Nash WJ (1992) An evaluation of egg-per-recruit analysis as a means of assessing size limits for blacklip abalone (*Haliotis rubra*) in Tasmania. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the world: biology, fisheries and culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 318-338.

Nash WJ, Sanderson JC, Bridley J, Dickson S, Hislop B (1995) Post-larval recruitment of blacklip abalone (*Haliotis rubra*) on artificial collectors in southern Tasmania. Marine and Freshwater Research 46:531-538.

Naylor JR, Andrew NL, Kim SW (2006a) Demographic variation in the New Zealand abalone *Haliotis iris.* Marine and Freshwater Research 57:215-224.

Naylor JR, McShane PE (1997) Predation by polychaete worms on larval and post-settlement abalone *Haliotis iris* (Mollusca: Gastropoda). Journal of Experimental Marine Biology and Ecology 214:283-290.

Naylor JR, McShane PE (2001) Mortality of post-settlement abalone *Haliotis iris* caused by conspecific adults and wave exposure. New Zealand Journal of Marine and Freshwater Research 35:363-369.

Naylor R, Neill K, Stewart R (2006b) Marine ecology - coralline algae and paua settlement. Water & Atmosphere 14:14-15.

Nielson LA (1992) Methods of marking fish and shellfish. American Fisheries Society Special Publication 23:208.

Officer RA, Dixon CD, Gorfine HK (2001) Movement and re-aggregation of the blacklip abalone, *Haliotis rubra* Leach, after fishing. Journal of Shellfish Research 20:771-779.

Olafsson EB, Peterson CH, Ambrose WG (1994) Does recruitment limitation structure populations and communities of macroinvertebrates in marine soft sediments - the relative significance of presettlement and postsettlement processes. In: Oceanography and Marine Biology, Vol 32. U C L Press Ltd, London, p 65-109.

Olla BL, Davis MW, Ryer CH (1994) Behavioural deficits in hatchery-reared fished: potential effects on survival following release. Aquaculture and Fisheries Management 25:19-34.

Olla BL, Davis MW, Ryer CH (1998) Understanding how the hatchery environment represses or promotes the development of behavioural survival skills. Bulletin of Marine Science 62:531-550.

Olsen DA (1968) Banding patterns in *Haliotis*. 2 - Some behavioural considerations and the effect of diet on shell coloration for *Haliotis rufescens*, *Haliotis corrugata*, *Haliotis sorenseni* and *Haliotis assimilis.* The Veliger 11:135-139.

Orensanz JM, Armstrong J, Armstrong D, Hilborn R (1998) Crustacean resources are vulnerable to serial depletion- the multifaceted decline of crab and shrimp fisheries in the Greater Gulf of Alaska. Reviews in Fish Biology and Fisheries 8:117-176.

Osumi K (1999) Stock enhancement and habitat association of juvenile paua (*Haliotis iris*) in northeastern New Zealand. Masters thesis, University of Auckland.

Peck LS, Culley MB (1990) Structure and density of *Haliotis tuberculata* populations around the coasts of Jersey, Channel-Isles. Journal of the Marine Biological Association of the United Kingdom 70:67-75.

Phillips NE, Shima JS (2006) Differential effects of suspended sediments on larval survival and settlement of New Zealand urchins *Evechinus chloroticus* and abalone *Haliotis iris.* Marine Ecology Progress Series 314:149-158.

Pirker JG, Schiel DR (1993) Tetracycline as a fluorescent shell-marker in the abalone *Haliotis iris.* Marine Biology 116:81-86.

Poore GC (1972) Ecology of New Zealand abalones, *Haliotis* species (Mollusca: Gastropoda) 2. Seasonal and diurnal movement. New Zealand Journal of Marine and Freshwater Research 6:246-258.

Preece PA, Shepherd SA, Clarke SM, Keesing JK (1997) Abalone stock enhancement by larval seeding: effect of larval density on settlement and survival. Molluscan Research 18:265-273.

Prince JD (2004) The decline of global abalone (Genus *Haliotis*) production in the late twentieth century: Is there a future? In: Leber KM, Kitada S, Blankenship HL, Svåsand T (eds) Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Science Ltd, Oxford, UK, p 427-443.

Prince JD, Ford WB (1985) Use of anaesthetic to standardize efficiency in sampling abalone populations (genus Haliotis: Mollusca: Gastropoda). Australian Journal of Marine and Freshwater Research 36:701-706.

Prince JD, Sellers TL, Ford WB, Talbot SR (1987) Experimental evidence for limited dispersal of haliotid larvae (genus *Haliotis*: Mollusca: Gastropoda). Journal of Experimental Marine Biology and Ecology 106:243-263.

Prince JD, Sellers TL, Ford WB, Talbot SR (1988a) Confirmation of a relationship between the localized abundance of breeding stock and recruitment for *Haliotis rubra* Leach (Mollusca: Gastropoda). Journal of Experimental Marine Biology and Ecology 122:91-104.

Prince JD, Sellers TL, Ford WB, Talbot SR (1988b) Recruitment, growth, mortality and population structure in a southern Australian population of *Haliotis rubra* (Mollusca, Gastropoda). Marine Biology 100:75-82.

Purcell SW, Blockmans BF, Agudo NNS (2006) Transportation methods for restocking of juvenile sea cucumber, *Holothuria scabra.* Aquaculture 251:238-244.

Raimondi PT, Wilson CM, Ambrose RF, Engle JM, Minchinton TE (2002) Continued declines of black abalone along the coast of California: are mass mortalities related to El Niño events? Marine Ecology Progress Series 242:143.

Roberts CM (1997) Ecological advice for the global fisheries crisis. Trends in Ecological Evolution 12:35-38.

Roberts R (2001) A review of settlement cues for larval abalone (*Haliotis* spp.). Journal of Shellfish Research 20:571-586.

Roberts RD, Kaspar HF, Barker RJ (2004) Settlement of abalone (*Haliotis iris*) larvae in response to five species of coralline algae. Journal of Shellfish Research 23:975-987.

Roberts RD, Kawamura T, Handley CM (2007a) Factors affecting settlement of abalone (*Haliotis iris*) larvae on benthic diatom films. Journal of Shellfish Research 26:323-334.

Roberts RD, Keys EF, Prendeville G, Pilditch CA (2007b) Viability of abalone (*Haliotis iris*) stock enhancement by release of hatchery-reared seed in Marlborough, New Zealand. Journal of Shellfish Research 26:697-703.

Roberts RD, Lapworth C (2001) Effect of delayed metamorphosis on larval competence, and post-larval survival and growth, in the abalone *Haliotis iris* Gmelin. Journal of Experimental Marine Biology and Ecology 258:1-13.

Roberts RD, Nicholson CM (1997) Variable response from abalone larvae (*Haliotis iris, H. virginea*) to a range of settlement cues. Molluscan Research 18:131-141.

Rogers-Bennett L, Pearse JS (1998) Experimental seeding of hatchery-reared juvenile red abalone in northern California. Journal of Shellfish Research 17:877-880.

Rosenberg AA (2003) Managing to the margins: the overexploitation of fisheries. Frontiers in Ecology and the Environment 1:102-106.

Rothlisberg PC, Preston NP (1992) Technical aspects of stocking: batch marking and stock assessment. In: Hancock DA (ed) Recruitment Processes Australian Society for Fish Biology Workshop, August 21, 1991. Bureau of Rural Resources Proceedings No. 16. Australian Government Printing Office, Canberra, p 187-191.

Rowley RJ, Mackinnon DI (1995) Use of the fluorescent marker calcein in biomineralisation studies of brachiopods and other marine organisms. Bulletin de l'Institut Oceanographique Numero Special (Monaco) 14:111-120.

Rudd M (1994) A review of international abalone trade patterns and pricing. Second International Symposium on Abalone Biology, Fisheries and Culture, Hobart, Tasmania, p 16.

Russ GR (2002) Yet Another Review of Marine Reserves as Reef Fishery Management Tools. In: Sale P (ed) Coral Reef Fishes. Elsevier Science, USA, p 421-441.

Sainsbury KJ (1982) Population-dynamics and fishery management of the paua, *Haliotis-iris*. I. Population-structure, growth, reproduction, and mortality. New Zealand Journal of Marine and Freshwater Research 16:147-161.

Saito K (1984) Ocean ranching of abalones and scallops in northern Japan. Aquaculture 39:1-4.

Sasaki R, Shepherd SA (1996) Recruitment processes of *Haliotis discus hannai* with special reference to larval distribution and settlement. In: Watanabe Y, Yamashita Y, Oozeki Y (eds) Survival Strategies in Early Life Stages of Marine Resources. A.A. Balkema, Brookfield, USA, p 325-340.

Sasaki R, Shepherd SA (2001) Ecology and post-settlement survival of the ezo abalone, *Haliotis discus hannai*, on Miyagi coasts, Japan. Journal of Shellfish Research 20:619-626.

Saunders T, Mayfield S (2008) Predicting biological variation using a simple morphometric marker in the sedentary marine invertebrate *Haliotis rubra*. Marine Ecology-Progress Series 366:75-89.

Schiel DR (1992) The enhancement of paua (*Haliotis iris* Martyn) populations in New Zealand. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 474-484.

Schiel DR (1993) Experimental evaluation of commercial-scale enhancement of abalone *Haliotis iris* populations in New Zealand. Marine Ecology Progress Series 97:167-181.

Schiel DR, Andrew NL, Foster MS (1995) The structure of subtidal algal and invertebrate assemblages at the Chatham Islands, New Zealand. Marine Biology 123:355-367.

Schiel DR, Welden BC (1987) Responses to predators of cultured and wild red abalone, *Haliotis rufescens*, in laboratory experiments. Aquaculture 60:173-188.

Schwarz CJ (2005) Estimation of movement from tagging data. In: Cadrin SX, Friedland KD, Waldman JR (eds) Stock Identification Methods: Applications in Fishery Science. Elsevier Academic Press, p 591-606.

Schwarz CJ, Seber GAF (1999) Estimating animal abundance: review III. Statistical Science 14:427-456.

Searcy-Bernal R (1999) Settlement and post-larval ecology of the red abalone *Haliotis rufescens* in culture systems. Doctorate of Philosophy, University of California, Davis and San Diego State University.

Searcy-Bernal R, Salas-Garza AE, Flores-Aguilar RA, Hinojosa-Rivera PR (1992) Simultaneous comparison of methods for settlement and metamorphosis induction in the red abalone (*Haliotis rufescens*). Aquaculture 105:241-250.

Searcy Bernal R, Anguiano Beltran C (1998) Optimizing the concentration of gamma-aminobutyric acid (GABA) for inducing larval metamorphosis in the red abalone Haliotis rufescens (Mollusca: Gastropoda). Journal of the World Aquaculture Society [J World Aquacult Soc] 29:p.463-470.

Seitz RD, Lipcius RN, Knick KE, Seebo MS, Long WC, Brylawski BJ, Smith A (2008) Stock enhancement and carrying capacity of blue crab nursery habitats in Chesapeake Bay. Reviews in Fisheries Science 16:329-337.

Seki T, Taniguchi K (2000) Rehabilitation of northern Japanese abalone, *Haliotis discus hannai*, populations by transplanting juveniles. In: Campbell A (ed) Workshop on rebuilding abalone stocks in British Columbia. Canadian Special Publication of Fisheries and Aquatic Sciences, p 72-83.

Shepherd SA (1973) Studies on southern Australian abalone (genus *Haliotis*). I. Ecology of five sympatric species. Australian Journal of Marine and Freshwater Research 24:217-257.

Shepherd SA (1985) Power and Efficiency of a Research Diver, with a Description of a Rapid Underwater Measuring Gauge: Their Use in Measuring Recruitment and Density of an Abalone Population. . In: Mitchell CT (ed) Diving for Science. American Academy of Underwater Science, La Jolla, CA, p 263-272.

Shepherd SA (1986a) Movement of the Southern Australian abalone *Haliotis laevigata* in relation to crevice abundance. Australian Journal of Ecology 11:295-302.

Shepherd SA (1986b) Studies on the southern Australian abalone (genus *Haliotis*). VII. Aggregative behaviour of *H. laevigata* in relation to spawning. Marine Biology 90:231-236.

Shepherd SA (1990) Studies on Southern Australian abalone (Genus *Haliotis*) XII. Long-term recruitment and mortality dynamics of an unfished population. Australian Journal of Marine and Freshwater Research 41:475-492.

Shepherd SA (1998) Studies on Southern Australian abalone (genus *Haliotis*). XIX. Long-term juvenile mortality dynamics. Journal of Shellfish Research 17:813-825.

Shepherd SA, Breen PA (1992) Mortality in abalone: its estimation, variability and causes. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 276-304.

Shepherd SA, Clarkson PS (2001) Diet, feeding behaviour, activity and predation of the temperate blue-throated wrasse, *Notolabrus tetricus*. Marine and Freshwater Research 52:311-322.

Shepherd SA, Daume S (1996) Ecology and Survival of Juvenile Abalone in a Crustose Coralline Habitat in South Australia. In: Watanabe Y, Yamashita Y, Oozeki Y (eds) Survival Strategies in Early Life Stages of Marine Resources. A.A. Balkema, Rotterdam, p 297-313.

Shepherd SA, Hearn WS (1983) Studies on Southern Australian abalone (Genus *Haliotis*). IV. Growth of *H. laevigata* and *H. ruber*. Australian Journal of Marine and Freshwater Research 34:461-475.

Shepherd SA, Laws HM (1974) Studies on southern Australian abalone (genus *Haliotis*). II. Reproduction of five species. Australian Journal of Marine and Freshwater Research 25:49-62.

Shepherd SA, Lowe D, Partington D (1992) Studies on Southern Australian abalone (genus *Haliotis*). XIII: Larval dispersal and recruitment. Journal of Experimental Marine Biology and Ecology 164:247-260.

Shepherd SA, Preece PA, White RWG (2000) Tired nature's sweet restorer? Ecology of abalone (*Haliotis* spp.) stock enhancement in Australia. In: Campbell A (ed) Workshop on Rebuilding Abalone Stocks in British Columbia. Canadian Special Publication of Fisheries and Aquatic Sciences, p 84-97.

Shepherd SA, Rodda KR (2001) Sustainability demands vigilance: evidence for serial decline of the greenlip abalone fishery and a review of management. Journal of Shellfish Research 20 (2):829-841.

Shepherd SA, Rodda KR, Vargas KM (2001) A chronicle of collapse in two abalone stocks with proposals for precautionary management. Journal of Shellfish Research 20:843-856.

Shepherd SA, Turner JA (1985) Studies on southern Australian abalone (genus *Haliotis*) VI. Habitat preference, abundance and predators of juveniles. Journal of Experimental Marine Biology and Ecology 93:285-298.

Simizu T, Uchino K (2004) Effects of extensive seeding on abalone, *Haliotis discus discus*, abundance on the pacific coast of Boso Peninsula, Japan. Journal of Shellfish Research 23:1209-1211.

Steinbeck JR, Groff JM, Friedman CS, McDowell T, Hedrick RP (1992) Investigations into a mortality among populations of the Californian black abalone, *Haliotis cracherodii*, on the central coast of California, USA. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 203-213.

Stekoll MS, Shirley TC (1993) In situ spawning behaviour of an Alaskan population of pinto abalone *Haliotis kamtschatkana* Jonas 1845. Veliger 36:95-97.

Stoner AW, Glazer RA (1998) Variation in natural mortality: Implications for queen conch stock enhancement. Bulletin of Marine Science 62:427-442.

Svåsand T, Kristiansen TS, Pedersen T, Salvanes AGV, Engelsen R, Naevdal G, Nodtvedt M (2000) The enhancement of cod stocks. Fish and Fisheries 1:173-205.

Sweijd N, Snethlage Q, Harvey D, Cook P (1998) Experimental abalone (*Haliotis midae*) seeding in South Africa. Journal of Shellfish Research 17:897-904.

Tarbath D, Mundy C, Haddon M (2005) Tasmanian Abalone Fishery 2004. Fishery Assessment Report, Tasmanian Aquaculture and Fisheries Institute

Tarr RJQ (1995) Growth and movement of the South African abalone *Haliotis midae*- a reassessment. Marine and Freshwater Research 46:583-590.

Tarr RJQ (2000) The South African abalone (*Haliotis midae*) fishery: A decade of challenges and change. In: Campbell A (ed) Workshop on rebuilding abalone stocks in British Columbia. Canadian Special Publication of Fisheries and Aquatic Sciences, p 32-40.

Tarr RJQ, Williams PVG, Mackenzie AJ, Plaganyi E, Moloney C (2000) South African fishery independent abalone surveys. Journal of Shellfish Research 19:537.

Tegner MJ (1992) Brood-stock transplants as an approach to abalone stock enhancement. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd., Oxford, UK, p 461-473.

Tegner MJ (1993) Southern California abalones: Can stocks be rebuilt using marine harvest refugia? In: Dugan JE, and Davis, G.E. (ed) International Symposium on Marine Harvest Refugia, p 2010-2018.

Tegner MJ (2000) Abalone (*Haliotis* spp.) enhancement in California: what we've learned and where we go from here. In: Campbell A (ed) Workshop on Rebuilding Abalone Stocks in British Columbia. Canadian Special Publication of Fisheries and Aquatic Sciences, p 61-71.

Tegner MJ, Basch LV, Dayton PK (1996) Near extinction of an exploited marine invertebrate. Trends in Ecological Evolution 11:278-280.

Tegner MJ, Butler RA (1985) The survival and mortality of seeded and native red abalones, *Haliotis rufescens*, on the Palos Verdes peninsula. California Fish and Game 71:150-163.

Tegner MJ, Butler RA (1989) Abalone Seeding. In: Hahn KO (ed) Handbook of Culture of Abalone and Other Marine Gastropods. CRC Press, Boca Raton, Florida, p 157-182.

Tegner MJ, DeMartini JD, Karpov KA (1992) The Californian red abalone fishery: a case study in complexity. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 370-383.

Tegner MJ, Ebert EE, Parker DO (1986) Abalone larval transplants as an approach to stock enhancement, University of California, San Diego. R/NP-1-15D

Tegner MJ, Haaker PL, Riser KL, Vilchis LI (2001) Climate variability, kelp forests, and the southern California red abalone fishery. Journal of Shellfish Research 20:755-763.

Thebault J, Chauvaud L, Clavier J, Fichez R, Morize E (2006) Evidence of a 2-day periodicity of striae formation in the tropical scallop *Comptopallium radula* using calcein marking. Marine Biology 149:257-267.

Thorrold SR, Jones GP, Hellberg ME, Burton RS, Swearer SE, Neigel JE, Morgan SG, Warner RR (2002) Quantifying larval retention and connectivity in marine populations with artificial and natural markers. Bulletin of Marine Science 70:291-308.

Tong LJ, Moss GA (1992) The New Zealand culture system for abalone. In: Shepherd SA, Tegner MJ, Guzman del Proo SA (eds) Abalone of the World: Biology, Fisheries and Culture. Blackwell Scientific Publications Ltd, Oxford, UK, p 583-591.

Tong LJ, Moss GA, Illingworth J (1987) Enhancement of a natural population of the abalone, *Haliotis iris*, using cultured larvae. Aquaculture 62:67-72.

Trapido-Rosenthal HG, Morse DE (1986) Regulation of receptor-mediated settlement and metamorphosis in larvae of a gastropod Mollusc (*Haliotis rufescens*). Bulletin of Marine Science 39:383-392.

Travis J, Coleman FC, Grimes CB, Conover D, Bert TM, Tringali M (1998) Critically assessing stock enhancement: an introduction to the Mote Symposium. Bulletin of Marine Science 62:305-311.

Treble RJ, Day RW, Quinn TJ (1993) Detection and effects on mortality estimates of changes in tag loss. Canadian Journal of Fisheries and Aquatic Sciences 50:1435-1441.

Tringali MD, Leber KM, Halstead WG, McMichael R, O'Hop J, Winner B, Cody R, Young C, Neidig C, Wolfe H, Forstchen A, Barbieri L (2008) Marine stock enhancement in Florida: A multi-disciplinary, stakeholder-supported, accountability-based approach. Reviews in Fisheries Science 16:51-57.

Troynikov VS, Day RW, Leorke AM (1998) Estimation of seasonal growth parameters using a stochastic Gompertz model for tagging data. Journal of Shellfish Research 17:833-838.

Underwood AJ (1997) Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Melbourne.

Underwood AJ, Kingsford MJ, Andrew NL (1991) Patterns in shallow subtidal marine assemblages along the coast of New South Wales. Australian Journal of Ecology 16:231-249.

van der Meeren GI (1991) Out-of-water transportation effects on behaviour in newly released juvenile Atlantic lobsters *Homarus gammarus.* Aquacultural Engineering 10:55-64.

Vosloo A, Vosloo D (2006) Routes of water loss in South African abalone (*Haliotis midae*) during aerial exposure. Aquaculture 261:670-677.

Wahle RA, Steneck RS (1991) Recruitment habitats and nursery grounds of the American lobster *Homarus americanus*- a demographic bottleneck. Marine Ecology Progress Series 69:231-243.

Ward RD (2006) The importance of identifying spatial population structure in restocking and stock enhancement programmes. Fisheries Research 80:9-18.

Welcomme RL (1998) Evaluation of stocking and introductions as management tools. In: Cowx IG (ed) Stocking and Introduction of Fish. Blackwell Science Ltd, Oxford, p 397-413.

Wells FE, Keesing JK (1990) Population characteristics of the abalone *Haliotis roei* on intertidal platforms in the Perth metropolitan area. Journal of the Malacological Society of Australia 11:65-71.

Werner I, Flothmann S, Burnell G (1995) Behaviour studies on the mobility of two species of abalone (*Haliotis tuberculata* and *H. discus hannai*) on sand: Implications for reseeding programmes. Marine and Freshwater Research 46:681-688.

Wertheimer AC, Heard WR, Smoker WW (2004) Effects of hatchery releases and environmental variation on wild-stock productivity: Consequences for sea ranching of Pink Salmon in Prince William Sound, Alaska In: Leber KM, Kitada S, Blankenship HL, Svåsand T (eds) Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Science Ltd, Oxford, UK, p 307-326.

White HI, Hecht T, Potgieter B (1996) The effect of four anaesthetics on *Haliotis midae* and their suitability for application in commercial abalone culture. Aquaculture 140:145-151.

Worthington D, Blount C (2003) Research to develop and manage the sea urchin fisheries of NSW and eastern Victoria. Final Report to Fisheries Research and Development Corporation. Project No. 1999/128

Worthington DG, Andrew NL (1997) Does covariation between growth and reproduction compromise the use of an alternative size limit for the blacklip abalone, *Haliotis rubra,* in NSW, Australia? Fisheries Research 32:223-231.

Worthington DG, Andrew NL (1998) Small-scale variation in demography and its implications for an alternative size limit in the fishery for blacklip abalone (*Haliotis rubra*) in New South Wales, Australia. Canadian Special Publication of Fisheries and Aquatic Sciences 125:341-348.

Worthington DG, Andrew NL, Hamer G (1995) Covariation between growth and morphology suggests alternative size limits for the blacklip abalone, *Haliotis rubra*, in New South Wales, Australia. Fishery Bulletin 93:551-561.

Worthington DG, Blount C, Chick RC, Gibson PT (1999) An assessment of the NSW abalone fishery in 1998. NSW Fisheries Fishery Resource Assessment Series No. 6, NSW Fisheries Research Institute, Cronulla, New South Wales, Australia.

Worthington DG, Chick RC, Blount C, Brett PA, Gibson PT (1998) A final assessment of the NSW abalone fishery in 1997. NSW Fisheries Fishery Resource Assessment Series No. 5, NSW Fisheries Research Institute, Cronulla, New South Wales, Australia.

Worthington DG, Chick RC, Blount C, Gibson PT, Stewart BR (2001) An assessment of the performance indicators of the Abalone Share Management Plan in 2000. NSW Fisheries Fishery Resource Assessment Series No. 11, NSW Fisheries Research Institute, Cronulla, New South Wales, Australia.